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COMPOSITION OF NORMAL AND MOTTLED CITRUS LEAVES[1]

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INTRODUCTION

Knowledge concerning the composition of a plant is essential to an understanding of its growth. The amounts and proportions of the different constituents absorbed from the soil or other nutrient medium, as revealed by accurate analysis of the several parts of plants, undoubtedly give some indication concerning their nutritional requirements. If determined progressively, such data may contribute to a clearer understanding of fundamental physiological processes of growth.

The interpretation of plant analyses, so far as growth processes and requirements are concerned, demands great caution, however. Many plants undoubtedly have the power of adapting themselves to a wide range of soil variations; and the composition of the plant, owing to selective absorption, commonly bears little direct relation to the composition of the nutrient solution. It is well known that the concentration of a given constituent in the nutrient solution may be varied considerably without producing any material change in the composition of the plant.

The effect of an excess or deficiency of one ion on the absorption of other ions, and especially the effects of nonessential salts on the absorption of essential ions, have not been sufficiently studied. Despite the many investigations during recent years on antagonism, comparatively few analyses have been made showing the effects on absorption. Likewise, investigations on the so-called nutritional or physiological diseases have not dealt with absorption specifically, except to a very limited extent.

Previous studies on the rate of absorption of nutrients have been conducted mainly with annual plants, chiefly cereals, very limited study having been devoted to trees. There is much need for accurate data on the several phases of absorption as related to the growth of fruit trees.

[1] Paper No. 67, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

In connection with investigations on the nutrition of different species of citrus trees, especially as related to that condition known as mottle-leaf, we have determined the composition of different parts of the tree, such, for example, as the roots, old wood, young wood, leaves, leaf sap, and fruit. This work has extended over a period of several years, and further study is contemplated. Some of the results already obtained have proved to be of special interest. The present paper will be devoted mainly to a discussion of the composition of the leaves.

It is not necessary to review the many published analyses of citrus fruits. Most of the publications on this subject have dealt mainly with the organic constituents and total ash, with an occasional analysis of the ash. Comparatively few analyses have been published showing the composition of portions of citrus trees other than the fruit.

The earliest investigation we have been able to find, and perhaps the best known, is that of Rowney and How (*15*)[1], published in 1848. Analyses were reported of the roots, stems, leaves, and fruit of orange trees, *Citrus aurantium*, grown on the island of St. Michael. The variety was presumably that now known as St. Michael.[2] The analyses were expressed as percentages of the carbon-dioxid-free ash. The results were similar to our analyses of California orange trees, when calculated to the same basis.

In 1891 Oliveri and Guerrieri (*13*) published an extended study on the composition of the wood, leaves, and different portions of the fruit of the orange, *Citrus aurantium* Riss;[2] Mandarin, *C. nobilis* var. *deliciosa*, Swingle; and lemon, *C. limonia* Osbeck, grown in Palermo, Italy. This investigation, extending over a period of three years, is the most complete study yet published on the composition of different parts of citrus trees. They recorded the number and weights of fruits produced by different classes of trees and the number and weights of leaves and the weights of wood pruned from the trees during a period of three years, representative samples of which were analyzed. Some of their analyses also agree reasonably closely with our data.

In 1901 Aliño (*1*) determined the phosphoric acid, potash, and nitrogen content of orange wood, leaves, and fruit; and in 1909 Muller (*12*) published complete analyses of seedling orange leaves from healthy and diseased trees grown in South Africa.

In 1910 Blair (*2*) analyzed orange leaves and stems grown in Florida. His samples represented the new growth taken in October from certain plots of a fertilizer experiment. In 1917 Jensen (7) published a paper on the composition of normal and mottled orange, lemon, and grape-

[1] Reference is made by number (italic) to "Literature cited," p. 190–191.

[2] In this case, the sweet orange, *Citrus sinensis* Osbeck, is doubtless the species studied. W. T. Swingle's revision of citrus nomenclature, as given in the "American Standard Cyclopedia of Horticulture," is followed in this paper.

fruit (*Citrus grandis* Osbeck) leaves grown in California. Further reference will be made to this paper later.

As is well known, the composition of annual herbaceous plants depends on their age. It has been shown that the ash content and the proportions of the individual constituents absorbed from the soil change as growth proceeds. Of the changes in perennials much less is known. It seems reasonable to suppose, however, that the growth processes are similar. The periodically developing new shoots may be likened to the portion of annual plants growing above ground.

New shoots appear on citrus trees several times each year. The tree, being evergreen, bears leaves at all seasons. Consequently, the foliage is composed of leaves of different ages. A given leaf ordinarily remains on the tree for a period of from two to three or more years.

SELECTION OF SAMPLES

Special care has been taken to secure representative samples of leaves of known age. Familiarity with the appearance of developing citrus leaves proved to be a material aid in selecting the samples. A considerable portion of the samples were obtained from trees growing near the laboratory where daily observations were made. The leaves of the Washington Navel and Valencia orange, the Eureka lemon, and the Marsh seedless grapefruit have been analyzed. Each sample was composed of several hundred leaves, collected from six or more adjacent trees, all of which were reasonably uniform in appearance and the culture and fertilization of which had been the same. The trees were 10 or more years of age. The entire leaf, including the petiole, was analyzed as a unit.

The samples were picked from the trees, placed in tight bags and immediately taken to the laboratory and weighed. In most cases this procedure did not require more than 30 minutes. In order to remove dust and other adhering foreign material, the leaves were thoroughly cleaned by wiping each leaf with a moist cloth, but washing with water was necessary with a few samples heavily coated with dust or showing evidences of residues from previous spraying. Early in this work it was found that the samples from which the dust had not been completely removed contained abnormally high percentages of silica, alumina, iron, and inorganic materials not soluble in dilute hydrochloric acid.

METHODS OF ANALYSIS

The samples were dried at 105° C. for 24 hours, and the loss in weight was calculated as moisture. The dry samples were ground to a powder in a small hand mill, were thoroughly mixed, and were then stored in tightly stoppered bottles for analysis.

Total nitrogen was determined by the official Kjeldahl method, modified to include nitrates. Total sulphur was determined by the sodium-peroxid

fusion method. The fusions were made over alcohol flames, and the sulphate was precipitated as barium sulphate, usually from the solution of the entire mass used in making the fusion. Total phosphorus was determined by treating 1 to 2 gm. of the dry material with a solution of magnesium nitrate, evaporating to dryness, igniting, and proceeding in the usual manner. Chlorin was determined in a special portion of the ash made by igniting at a low heat 5 to 10 gm. of the dry material, dissolving the residue in dilute nitric acid, and proceeding with the Volhard volumetric method. In some cases chlorin was also determined by performing the incineration in the presence of an excess of sodium carbonate in order to avoid the possible loss of chlorin, but the results of the two methods were similar.

For the determination of total ash, 10 to 20 gm. of the dry samples were incinerated in porcelain dishes over Bunsen burners. The material charred easily and burned quietly upon the application of low heat and was reduced to a gray ash without approaching dull redness. The residue was then allowed to cool, was taken up with hot water, transferred to a filter, and washed thoroughly. The insoluble material with its filter paper was transferred to a platinum dish, dried, pulverized with an agate pestle, and heated to full redness. When the platinum dish cooled, the filtrate from the previous leaching was added and evaporated to dryness. Ten to 20 cc. of strong ammonium-carbonate solution were then added, and the treatment was repeated until the ash was completely carbonated, as was indicated by constant weight upon evaporating to dryness and heating gently. The results are recorded as percentages of ash. It should be stated that the ash thus obtained differs from that reported by other investigators in that we are dealing with completely carbonated ash, whereas previous analyses of citrus leaf ash have been calculated to a carbon-dioxid-free basis.

The ash was dissolved in water and dilute hydrochloric acid, and the solution was evaporated to complete dryness on the water bath in order to dehydrate the silica. The amount of uncombined carbon found in the ash was always entirely negligible. The residue was taken up with warm water and dilute hydrochloric acid. The silica was determined by the loss in weight occasioned by treating the incinerated residue with hydrofluoric acid. The material nonvolatile in hydrofluoric acid usually amounted to only 0.1 to 0.2 per cent of the ash and was neglected in this work. The filtrate from the silica separation was made up to a definite volume, usually 500 cc., and the various constituents were determined in aliquots representing from 0.2 gm. to 0.4 gm. of the ash.

The methods of the Association of Official Agricultural Chemists[1] were used with slight modifications, as noted. Iron, aluminum, and

[1] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig., 1908. Reprinted in 1912.

phosphoric acid were precipitated collectively by adding a weighed excess of ferric chlorid, neutralizing with ammonia, filtering, redissolving in dilute hydrochloric acid, and repeating the process. Iron was precipitated with ammonia from a separate aliquot and determined volumetrically by reduction with zinc and titration with permanganate. This method was occasionally supplemented by the ferrocyanid colorimetric method with fairly satisfactory results. Aluminum was calculated by difference after the phosphoric acid was gravimetrically determined in a separate aliquot. Calcium, magnesium, potassium, and sodium were determined in the filtrate after the ammonia precipitate was removed, and in some cases manganese was determined by bromin oxidation. Sulphate was determined gravimetrically in an aliquot of the original solution. Carbon dioxid was not determined.

COMPOSITION OF NORMAL MATURE ORANGE LEAVES

A considerable number of analyses have been made of mature orange leaves representing both the Washington Navel and Valencia varieties. Owing to the absence of previous records showing the age of the leaves available for analysis, and in view of the fact that orange leaves, when from 4 to 6 months of age, assume an appearance not unlike that of leaves 1, 2, or more years of age, it is highly probable that random samples will always represent mixed ages.[1] Most of our samples of mature leaves were taken at random, always avoiding immature or abnormal individuals. The samples were gathered at different seasons of the year and from a considerable number of different sets of trees, some of which were growing in different localities. Typical analyses are submitted in Tables I and II.

It is interesting to note that the composition of the different samples was found to be reasonably uniform despite the fact that their average ages, although they were mature in appearance, probably varied considerably. Other samples not reported above showed a similar composition. The data also afford but little evidence of seasonal variation in composition.

Except in calcium and potassium content, the different samples of the same variety differed almost as widely in composition as the samples of different varieties. The samples from different localities were also similar in composition, although those from Riverside were grown on sandy loam soil, that from Anaheim on light sandy soil, and the one from Whittier on heavy adobe.

It will be noted that the average calcium content of Valencia leaves was found to be somewhat higher than that of Navels, while the reverse is true for potassium.

[1] Ensign (6) has recently shown that the size of the vein islets of *Citrus grandis* is directly correlated with the maturity of the leaf. From the most immature to fully matured leaves there is a gradual increase in the size of the vein islets. If further investigation prove that similar relations occur in other species of citrus, a direct means will be afforded by which the age of the leaves can be determined.

TABLE I.—*Composition of mature normal orange leaves*

Locality.	Date collected.	Probable age (in months).	Constituents of ash.								
			SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
NAVEL			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot U	Dec. 12, 1916	6 to 24	2.17	0.14	30.68	1.53	7.33	0.83	2.83	2.85	0.24
Riverside, plot V	do	do	2.54	.18	31.67	1.84	5.13	1.07	2.80	3.65	.39
Riverside, plot U	May 21, 1917	6 to 12	1.54	.14	31.89	1.81	6.67	.43	2.27	2.25	1.13
Average			2.08	.15	31.41	1.73	6.38	.78	2.63	2.92	.95
VALENCIA											
Arlington	Jan. 29, 1917	6 to 24	2.94	0.18	33.79	2.82	4.11	0.90	2.46	2.52	0.24
Riverside, plot U	May 23, 1917	6 to 12	2.25	.10	34.40	1.75	3.33	.48	1.97	2.47	.56
Whittier	Feb. 1, 1917	6 to 24	1.17	.07	35.58	1.04	3.68	.40	2.16	2.64	.15
Anaheim	Aug. 29, 1917	12 to 24	3.22	.11	32.97	1.32	5.45	.62	1.76	2.46	.19
Average			2.39	.11	34.19	1.88	4.74	.60	2.09	2.52	.28

TABLE II.—*Composition of mature normal orange leaves*

Locality.	Date collected.	Probable age (in months).	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
					SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
NAVEL			*Per cent.*	*Per cent.*										
Riverside, plot U	Dec. 12, 1916	6 to 24	59.58	15.39	0.34	0.021	4.73	0.24	1.13	0.13	0.14	0.45	2.70	0.04
Riverside, plot V	do	do	58.86	15.23	.39	.028	4.82	.28	.78	.16	.13	.42	2.58	.06
Riverside, plot U	May 21, 1917	6 to 12	62.35	16.33	.25	.022	5.21	.23	1.09	.07	.14	.25	2.12	.18
Average			60.26	15.65	.33	.024	4.92	.27	1.00	.12	.14	.37	2.47	.09
VALENCIA														
Arlington	Jan. 29, 1917	6 to 24	55.40	14.38	0.42	0.026	4.86	0.21	0.59	0.13	0.11	0.30	2.31	0.03
Riverside, plot U	May 23, 1917	6 to 12	61.49	16.45	.37	.017	5.66	.29	.55	.08	.17	.27	2.02	.09
Whittier	Feb. 1, 1917	6 to 24	58.77	18.25	.21	.013	6.49	.30	.67	.07	.20	.36	2.41	.03
Anaheim	Aug. 29, 1917	12 to 24	63.49	18.90	.61	.021	6.23	.25	1.03	.12	.18	.34	2.23	.03
Average			59.79	16.99	.40	.019	5.81	.26	.71	.10	.16	.32	2.24	.04

Throughout this work we have determined the aluminum. Qualitative tests usually indicated this element to be present, but the quantity was never more than a few tenths of 1 per cent of the ash. Frequently the amount was undeterminable. The manganese was also determined in several samples. The amount was found to vary from 0.1 per cent to 0.2 per cent of the ash.

The size of the leaves as gauged by their average weights was recorded, but there appears to be no consistent difference in composition referable to the size of the leaf. As is well known, the size of apparently normal orange leaves may vary widely. Even on a given tree, the fully mature leaves of certain cycles of growth may be at least twice as large as others.

From the analysis of many other samples in this laboratory it may be said that the composition of mature orange leaves when grown in California is remarkably uniform, provided, however, that the leaves be borne on vigorous trees. On the other hand, the composition of the leaves of improperly nourished and diseased trees may vary widely. If the supply of available nitrate be deficient, the content of nitrogen in the leaves may be considerably below that reported above, but there seems to be some doubt whether the reverse is true.

COMPOSITION OF LEMON AND GRAPEFRUIT LEAVES

The analysis of mature Eureka lemon and Marsh seedless grapefruit leaves is submitted in Tables III and IV.

Two of the samples of lemon leaves were collected in midwinter and the other on August 29. They were grown on widely different types of soil. The Riverside sample grew on sandy loam, the Whittier sample on heavy adobe, and the Tustin sample on highly calcareous sandy loam soil. The grapefruit leaves were grown on sandy loam.

The composition of the different samples of lemon leaves is fairly uniform, the average being similar to the average composition of Valencia orange leaves. On the other hand, the composition of the grapefruit leaves closely resembles that of Navel orange leaves.

The composition of the leaves of the different varieties and species of citrus has been found to be remarkably uniform from the standpoint of both the ash and the dry matter. A more detailed discussion of the composition will be given below.

TABLE III.—*Composition of mature lemon and grapefruit leaves*

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
LEMON										
Riverside, plot U	Jan. 30, 1917	3.07	0.08	32.46	1.73	3.96	0.67	2.13	2.48	0.15
Whittier	Feb. 1, 1917	1.21	.07	34.02	2.55	4.63	.28	3.27	3.82	.22
Tustin	Aug. 29, 1917	1.02	.07	33.16	1.44	6.00	.30	2.12	2.43	.16
Average		.77	.07	33.21	1.91	4.86	.42	2.51	2.91	.18
GRAPEFRUIT										
Riverside	Dec. 10, 1917	2.18	0.09	31.25	2.31	5.96	0.25	3.21	2.99	0.12

TABLE IV.—*Composition of mature lemon and grapefruit leaves*

Locality.	Date collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
		Per cent.	*Per cent.*										
LEMON													
Riverside, plot U	Jan. 30, 1917	58.35	18.21	0.56	0.014	5.91	0.32	0.72	0.12	0.16	0.29	2.10	0.03
Whittier	Feb. 1, 1917	56.67	15.12	.18	.010	5.14	.39	.71	.04	.20	.38	2.65	.03
Tustin	Aug. 8, 1917	61.42	17.67	.18	.012	5.86	.25	1.06	.05	.16	.29	2.28	.03
Average		58.81	17.00	.31	.012	5.64	.32	.83	.07	.17	.32	2.34	.03
GRAPEFRUIT													
Riverside	Dec. 10, 1917	59.39	17.65	0.38	0.016	5.51	0.41	1.06	0.04	0.21	0.22	1.98	0.02

COMPOSITION OF ORANGE LEAVES AT DIFFERENT STAGES OF GROWTH

The results obtained from the analysis of samples of leaves approximately one month of age, gathered on May 11, 1917, were found to be considerably different from previous analyses of mature leaves. Samples representing the new spring growth and that of the previous year, gathered from the same trees on May 21, 1917, also proved to be widely different in composition. These results, together with the discordance between the analyses previously made in this laboratory and those published by Blair (2) from Florida and by Jensen (7) from California, suggested the desirability of making a study on the composition of orange leaves at different stages of growth.

Samples were collected at four different intervals in the growth cycle. The first represented leaves approximately 1 week old; the second, those 6 to 8 weeks old; the third, leaves at full maturity, the ages of which ranged from 6 months to approximately 2 years; the fourth, old leaves that were about to be shed, as indicated by their yellowish brown color. Each sample was picked from six normal, vigorously growing trees of plot V at the Citrus Experiment Station, Riverside, Calif. The samples representing different ages were all taken from the same trees, and those representing the first three periods of growth were gathered on the same day, November 9, 1917. These trees support an abundant foliage; and, as frequently occurs, they at that time bore numerous shoots of varying ages, ranging from a few days to 2 or more years of age, which made it possible to secure samples of widely different ages on a given day. The samples of old leaves were gathered December 10, 1917.

The data expressed as percentages of the ash show that notable changes take place in the relations of certain constituents as growth proceeds. Especially prominent among these changes are the decreases in the percentages of phosphate and potassium, on the one hand, and the increases in calcium on the other. For example, the ash of navel leaves at the age of 1 week was found to contain 16.83 per cent phosphate (PO_4), at 6 weeks 7.10 per cent, at maturity 2.47 per cent, while the ash of old leaves contained only 1.32 per cent.

The changes in the percentages of potassium were quite parallel to those of phosphate. When navel leaves were 1 week of age, the ash contained 19.87 per cent potassium, when 6 weeks of age, 10.32 per cent, when mature, 5.68 per cent, while the old leaves contained only 1.66 per cent.

The percentages of calcium underwent changes quite opposite to those of potassium. With the ash containing 20.72 per cent calcium when the leaves were 1 week old there was an increase to 28.44 per cent at 6 weeks, to 33.21 per cent at maturity, and finally to 34.41 per cent in the very old stage.

TABLE V.—*Composition of orange leaves at different stages of growth*

NAVEL

Age of leaves.	Constituents of ash.								
	SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1 week	1.82	0.28	20.72	3.91	19.87	1.20	16.83	5.88	0.32
6 weeks	.87	.17	28.44	3.29	10.32	.57	7.10	5.74	.15
Mature, 6 months to 2 years	1.70	.12	33.21	2.20	5.68	.46	2.47	3.51	.27
Very old, 3 or more years	1.96	.07	34.41	1.67	1.66	.44	1.32	3.09	.40

VALENCIA

1 week	2.42	0.24	19.82	3.49	19.45	1.35	14.28	5.30	1.04
6 weeks	1.10	.14	27.24	2.89	12.66	.74	7.44	4.42	.58
Mature, 6 months to 2 years	1.13	.13	32.90	2.08	5.93	.58	2.79	3.22	.78
Very old, 3 or more years	1.11	.07	34.49	1.71	1.88	.42	1.49	1.94	1.31

TABLE VI.—*Composition of orange leaves at different stages of growth*

NAVEL

Age of leaves.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
			SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
	Per cent.	*Per cent.*										
1 week	72.31	6.54	0.12	0.018	1.36	0.26	1.30	0.08	0.36	0.25	3.01	0.02
6 weeks	70.81	9.20	.08	.016	2.62	.30	.95	.05	.21	.27	2.45	.01
Mature, 6 months to 2 years	60.98	16.95	.29	.021	5.63	.37	.96	.08	.13	.36	2.39	.05
Very old, 3 or more years	60.73	21.39	.42	.016	7.36	.36	.36	.09	.14	.37	1.31	.08

VALENCIA

1 week	72.69	7.53	0.18	0.018	1.49	0.26	1.46	0.10	0.35	0.25	3.03	0.08
6 weeks	68.54	9.12	.10	.013	2.48	.26	1.16	.07	.22	.28	2.53	.05
Mature, 6 months to 2 years	60.52	16.35	.18	.021	5.39	.34	.97	.09	.15	.28	2.37	.13
Very old, 3 or more years	59.19	22.32	.25	.016	7.70	.38	.42	.09	.13	.30	1.35	.29

Among the other necessary nutrients, the percentages of iron, magnesium, and sulphate decreased with age, although to a lesser degree than potassium and phosphate. The ash of the youngest leaves contained approximately twice as much iron as that of the mature leaves, and differences almost as great occurred in the percentages of magnesium and sulphate.

As was anticipated, the changes that take place in Valencia orange leaves are quite similar to those of navel leaves.

The percentages of phosphorus and sulphur refer to the total amounts as determined by the magnesium-nitrate and sodium-peroxid fusion methods, respectively, and are somewhat higher than the corresponding data calculated from the ash analyses. As is well known, organic materials usually lose a portion of their phosphorus and sulphur in the ashing process.

It will be noted that the content of water decreased considerably as growth took place. At 1 week of age the navel leaves contained 72.31 per cent water, at 6 weeks 70.81 per cent, at maturity 60.98 per cent, and the very old leaves still contained 60.73 per cent. The content of total ash, on the other hand, increased markedly with age, rising from 6.54 per cent of the dry matter at the age of 1 week to the very high content of 21.39 per cent in the old leaves.

The nitrogen decreased from 3.01 per cent at the age of 1 week to 2.39 per cent at maturity, and finally to 1.31 per cent in the old stage. The percentage of phosphorus decreased still more rapidly during the actively growing period, but later the phosphorus content remained approximately constant. The percentage of potassium also decreased rapidly during the early period of growth but remained almost constant after the second period until the period of senility approached, when a still further decrease took place.

The percentage of iron in the dry matter was found to be reasonably constant at all stages of growth. However, in considering the iron content of these and all other samples reported herein, it is important to bear in mind that the analytical error involved in the determination of small amounts of this element is likely to be relatively great. For this reason small variations in the results are probably not significant. The percentages of sulphur and magnesium each increased somewhat as growth took place.

The constituent of the dry matter of orange leaves that undergoes the greatest percentage change as a result of growth is calcium. At 1 week of age, the navel leaves contained 1.36 per cent calcium, at 6 weeks 2.62 per cent, at maturity 5.63 per cent, and the very old leaves contained 7.36 per cent.

Of the supposedly unessential constituents, the greatest concentration of sodium was found in the young leaves; but the amount was always small, while the data for silica and chlorin show no consistent variation.

It is interesting to note that in certain respects the composition of orange leaves changes with growth, somewhat as is the case with the vegetative portion of other plants. With certain cereals a considerable portion of the potassium, magnesium, phosphorus, and nitrogen migrate from the leaves into other parts of the plant as maturity approaches (*9*, *10*). The potassium tends to accumulate in the straw of rice, while the magnesium, phosphorus, and nitrogen are translocated to the grain.

The composition of citrus leaves differs markedly from that of cereals in certain other respects. The ash content of the former increases much more rapidly and reaches a very high point in the old leaves. The calcium content increases very rapidly during the most actively growing period and continues to be deposited in the leaves, although at a somewhat slower rate, almost until the time the leaves fall off.

While it is probable that the composition of normal orange leaves varies to some extent when grown in different parts of the world or on different soils in a given locality, careful study of the analyses of the Florida-grown leaves published by Blair (*2*) and those reported from Italy by Olivieri and Guerrieri (*13*) suggests that these were immature leaves. From Jensen's results (*7*), it is evident that his samples were not composed of mature leaves. Recognition of the relationships between the age and the composition of orange leaves is especially important in the study of the composition of mottled leaves, as will be pointed out more fully later.

It does not necessarily follow from the preceding discussion that a portion of a given element, potassium, for example, migrates back into other parts of the tree after the leaves reach a certain stage of development. Increase in the size of a leaf, owing to the elaboration of carbonaceous matter, may dilute the nutrients present and, therefore, lower the percentage without there being an actual loss. To establish this point, it is necessary to determine the weights of the constituents present per leaf at different periods. From the average weights of the individual leaves at each period we have calculated the content of the different constituents, expressing the results in grams per 1,000 leaves. (Table VII.)

The old Navel leaves were considerably smaller on the average than either those representing maturity or 6 weeks of age, while the mature Valencia leaves were larger than the old leaves of the same variety. In addition, the leaves of each sample of the Valencia variety were considerably larger than the corresponding Navel leaves.

Despite these irregularities in the size of the leaves, the data show that the content of calcium in a given orange leaf increases very rapidly during the early part of the growth period. In the Navel leaves, approximately a tenfold increase in calcium content took place between the first and the sixth week of age. From the sixth week to maturity a further increase, more than twofold, took place, and finally the calcium content increased still further as the leaves approached the time of normal dropping.

TABLE VII.—*Average amounts of constituents in 1,000 normal orange leaves*

Age of leaves.	Fresh material.	Dry matter.	Ca.	Mg.	K.	P.	N.	S.
	NAVEL							
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1 week	227	62.80	0.85	0.16	0.82	0.23	1.89	0.16
6 weeks	1.094	319.30	8.36	.96	3.03	.67	7.82	.86
Mature	852	322.03	18.13	1.19	3.09	.42	7.70	1.16
Very old	756	297.00	21.87	1.07	1.07	.41	3.89	1.10
	VALENCIA							
1 week	356	97.30	1.45	0.25	1.42	0.34	2.94	0.24
6 weeks	1.183	372.22	9.23	.97	4.32	.82	9.42	1.04
Mature	1.080	426.29	22.98	1.44	4.13	.64	10.10	1.19
Very old	801	327.70	25.23	1.24	1.38	.43	4.42	.98

The rates of increase in magnesium and sulphur are also rapid during the early part of the growth period, and each of these constituents continues to accumulate in the leaves up to maturity, but the absolute amounts never become high. Since irregularities occurred in the size of the leaves, it is doubtful whether any important amount of either magnesium or sulphur is translocated to other portions of the tree after maturity has been reached.

The maximum amounts of potassium, phosphorus, and nitrogen were deposited before the leaves were 6 weeks of age. The rates of increase of each were considerably less than that of calcium. The data show that a considerable portion of these elements migrates away from the leaves after certain periods. With potassium and nitrogen the loss takes place after maturity has been reached, while the phosphorus begins to recede even before maturity is attained.

Similar data for iron are omitted because of the magnitude of the analytical error involved in its determination.

Samples representing more frequent intervals in the growth cycle would certainly afford more detailed information regarding absorption. It is possible that the analysis of such samples when plotted might show breaks in the curves not indicated by the existing data. For example, the exact period in the growth cycle when the leaves contained the maximum amount of potassium might be shifted to some extent and other fluctuations might also be found. However, other analyses of immature orange leaves at different seasons of the year show a fairly close agreement with those reported above. On the whole, we are inclined to believe that the main features of the composition of the orange leaf have been determined.

It seems appropriate to emphasize the fact that citrus leaves are extremely calcareous, and much more so than most of the economic plants. As is well known, the ash of some of the legumes contains high percentages of calcium, but relatively few have been reported to contain as high percentages of calcium as citrus leaves. Not only is the ash of citrus leaves high in calcium but the total ash content is high also. It is unusual to find dried plant material that contains from 5 to 7 per cent calcium.

COMPOSITION OF MOTTLED ORANGE LEAVES

The condition of citrus trees known as mottle-leaf has been widely discussed. Much study has already been devoted to it, and several hypotheses have been advanced concerning the disease. The symptoms, mode of occurrence, and general distribution were fully discussed in a paper by Briggs, Jensen, and McLane (3). The disease is commonly thought to result from some nutritional disturbance, but the cause has not been definitely determined.

TABLE VIII.—*Composition of mottled orange leaves*

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
NAVEL		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot H	Mar. 19, 1915	3.10	0.16	24.27	2.04	13.13	1.92	4.91	4.86	0.26
Riverside, plot A	Dec. 9, 1916	1.73	.16	23.03	2.12	15.87	1.04	6.44	4.93	.31
Do	do	1.44	.14	23.38	2.42	13.40	1.65	7.16	4.42	.34
Do	May 21, 1917	1.80	.13	20.24	2.47	12.22	.70	5.56	4.74	.67
Average		2.02	.15	24.23	2.26	13.65	1.33	6.02	4.74	.39
VALENCIA										
Riverside, plot A	June 13, 1916	4.07	0.14	27.76	2.40	10.22	0.90	4.47	4.72	0.61
Riverside, plot H	do	3.74	.12	29.33	2.29	8.11	.63	4.43	4.51	.39
Arlington	Jan. 29, 1917	2.15	.13	25.77	3.70	13.81	.81	6.89	4.40	.24
Riverside, plot H	May 23, 1917	1.84	.10	28.37	2.54	9.22	.65	5.27	3.98	.61
Average		2.95	.12	27.81	2.73	10.34	.75	5.26	4.40	.46

TABLE IX.—*Composition of mottled orange leaves*

Location.	Date collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
NAVEL		*Per cent*	*Per cent.*										
Riverside, plot H	Mar. 19, 1915	63.75	15.15	0.47	0.035	3.68	0.31	1.99	0.29	0.29	0.37	3.19	0.04
Riverside, plot A	Dec. 9, 1916	64.84	12.36	.21	.020	2.85	.26	1.96	.13	.25	.46	3.12	.04
Riverside, plot H	do	63.57	13.64	.20	.019	3.19	.33	1.83	.22	.33	.59	3.60	.05
Do	May 21, 1917	65.22	14.80	.27	.019	3.88	.37	1.81	.10	.28	.36	3.91	.10
Average		64.34	13.99	.29	.021	3.40	.32	1.90	.18	.29	.42	3.45	.06
VALENCIA													
Riverside, plot A	June 13, 1916	62.16	13.94	0.57		3.87	0.33	1.42	0.13	0.17	0.38	2.59	0.06
Riverside, plot H	do	62.50	15.98	.60	0.019	4.69	.36	1.29	.10	.25	.37	3.03	.06
Arlington	Jan. 29, 1917	66.83	11.70	.25	.015	3.01	.22	1.62	.09	.25	.30	3.21	.09
Riverside, plot H	May 23, 1917	62.87	15.07	.28	.014	4.27	.38	1.39	.10	.28	.33	3.57	.08
Average		63.59	14.17	.45	.016	3.96	.32	1.43	.10	.24	.34	3.10	.07

We have analyzed different portions of orange and lemon trees affected with mottle-leaf, as well as grapefruit leaves and samples representing different degrees of mottling. Most of the samples were collected from the fertilizer plots of the Citrus Experiment Station. In all cases the leaves were collected from shoots 6 or more months of age. The analysis of orange leaves in an advanced stage of mottling is presented in Tables VIII and IX.

Comparison of the data with the previously submitted analyses shows at once that the composition of mottled leaves differs considerably from that of average mature normal leaves. The principal differences are found in the greater percentages of potassium and phosphate, on the one hand, and the lesser percentages of calcium on the other. The ash of mottled leaves also contains greater percentages of magnesium and sulphate, while the iron, silica, sodium, and chlorin do not differ materially.

Considerable variations will also be noted among the different samples of mottled leaves. This is probably due to the varying degrees of mottling represented by the samples. However, every sample of mottled leaves that has been analyzed in this laboratory has been found to vary from the normal in the same general direction.

The average content of water in mottled leaves was found to be slightly higher than in normal leaves and the ash content somewhat lower. Considering the dry matter, the most pronounced differences are found in the lesser calcium content, on the one hand, and the abnormally high percentages of potassium and phosphorus in mottled leaves, on the other. The average nitrogen content of mottled leaves is also considerably above normal, as was previously pointed out by McBeth (*11*).

From his analyses of normal and mottled citrus leaves, Jensen (*7*) failed to find any consistent difference in composition. In order to insure uniformity in the age of his samples, he collected the leaves from the current season's growth. On the dates two of his samples were collected, April 18 and May 11, the current season's growth is probably never mature at Riverside. Furthermore, the calcium content, which he reported, was very much below that of any mature normal orange leaf we have been able to find. It seems safe to conclude, therefore, that Jensen's studies were made with immature leaves. It is possible, of course, that the variations in composition incident to mottling may not occur until after the leaves have reached a certain stage of growth, although recent analysis of a sample of leaves about 10 days of age, taken from severely mottled trees, indicates that the composition may begin to diverge from the normal at a very early period.

It is well known that, with the exception of severe cases of mottle-leaf, the discoloration ordinarily does not become apparent until the leaves have reached an age of 2 to 3 months. Subsequently, the degree of discoloration becomes increasingly intense until the period of normal

maturity. In addition, mottle-leaf is usually most pronounced from September to February, when it becomes very noticeable on the leaves of the previous spring and summer cycles of growth.[1]

Some light may be thrown on mottle-leaf by comparing the composition of mottled leaves with that of normal leaves at different stages of growth. By reference to Tables V and VIII it will be seen that the composition of the ash of the former is quite similar to that of normal leaves approximately 6 weeks of age, although the total ash content of mottled leaves is considerably higher (compare Tables VI and IX). It is especially interesting to note that the nitrogen content of mottled leaves is somewhat higher than that of normal leaves at the age of 1 week and much greater than that of normal leaves at the age of 6 weeks.

The data indicate, therefore, that the essential nutrients are deposited in mottled orange leaves at abnormal rates. A satisfactory explanation of this fact can not now be given. The rising sap is itself probably abnormal in composition.

By calculating the weights of the several constituents contained in a unit number of mottled leaves, it is found (Table X) that notwithstanding the fact that the average size of the mottled leaves was less than one-half that of normal leaves they contained as great amounts of potassium and approximately as much phosphorus per leaf (compare Tables VII and X). On the other hand, the content of calcium was less than one-third as great as normally occurs, while the magnesium, sulphur, and nitrogen were intermediate in amount.[2]

The preceding analyses represent extreme cases of mottling. Samples of Valencia orange leaves at a less advanced stage have also been studied. These latter were of an intermediate size, showing the typical yellowish spots between the veins. They were selected from trees a considerable portion of whose foliage was normal and some of which bore a fair crop of fruit. The results are recorded in Table XI

The percentages of calcium and potassium closely approach those of severely mottled Valencia leaves (Tables VIII and IX), but the phosphorus content is more nearly normal. The percentage of nitrogen was found to be no greater than occurs in normal Valencia leaves.

Thus, it appears that the early stages of mottling are first attended by the absorption of subnormal amounts of calcium [3] and supernormal amounts of potassium and phosphorus, and that modifications in the absorption of nitrogen occur later.

[1] Mottled leaves fall off in large numbers during the latter part of the winter and early spring. New shoots developing at this season give the trees the appearance of having recovered from the disease. These latter, however, may become mottled the following fall. It is never safe to pass judgment on the state of the disease in the spring or early summer. We have never known of a leaf once severely mottled which became normal later. New leaves grown later, however, may be entirely normal.

[2] These data were calculated for only a portion of the samples of mottled leaves, because the average weight of the leaves was not determined for all the samples.

[3] Jensen (7) found that the yellow spots of mottled orange leaves, similar to those discussed here, contain less calcium than the remaining portion of the leaf.

TABLE X.—*Average amounts of constituents in 1,000 mottled orange leaves*

Locality.	Fresh material.	Dry matter.	Ca.	Mg.	K.	P.	N.	S.
				NAVEL				
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Riverside, plot A	480	169	4.81	0.44	3.31	0.42	5.27	0.78
Riverside, plot H	521	189	6.03	.62	3.46	.62	6.80	.94
Riverside, plot H	425	148	5.74	.55	2.68	.31	5.78	.53
Average	475	169	5.53	.54	3.15	.45	5.85	.75
				VALENCIA				
Arlington	755	250	7.52	0.55	4.05	0.62	8.02	0.75
Riverside, plot H	352	130	5.55	.49	1.90	.36	4.64	.43
Average	553	190	6.53	.52	2.97	.49	6.33	.59

TABLE XI.—*Composition of Valencia orange leaves at intermediate stages of mottling*

Locality.	Date.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Escondido	Feb. 1, 1917	2.67	0.13	28.74	2.13	11.38	0.82	4.18	2.80	0.44
Riverside, plot R	May 29, 1917	2.83	.06	30.55	2.00	7.48	1.18	3.23	3.74	.40
Riverside, plot U	June 13, 1916	.....	.....	28.75	1.91	9.16	.90	6.44	4.26	.26
Average		.....	.....	29.36	2.01	9.34	.97	4.62	3.60	.37

Locality.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
			SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
	Per cent.	*Per cent.*										
Escondido	60.72	13.58	0.36	0.018	3.90	0.14	1.54	0.11	0.17	0.27	2.53	0.06
Riverside, plot R	60.47	14.12	.40	.009	4.31	.28	1.05	.17	.18	.32	1.77	.06
Riverside, plot U	62.07	13.51	.55	.....	3.89	.41	1.24	.12	.22	.32	2.36	.03
Average	61.09	13.74	.44	.....	4.03	.28	1.28	.13	.19	.30	2.22	.05

Severely mottled lemon and grapefruit leaves have also been analyzed (Tables XII and XIII).

The results show that the composition of mottled lemon and grapefruit leaves is similar to that of mottled orange leaves. As was found from the analysis of normal leaves, the composition of lemon leaves closely resembles that of Valencia orange leaves, while the composition of grapefruit leaves was found to be like that of Navel leaves. However, the different varieties and species do not vary greatly in composition.

The fact that the composition of the leaves of one species of citrus is affected in the same general way as that of other species is not surprising, since their appearance when mottled is also similar.

As is well known, it is rare that all the leaves on a given orange tree are mottled. As a rule, those growing on the outer portions of the tree are the most severely affected, as sometimes, although not invariably, is the case with the leaves borne on the south and southeastern portion of the trees. The leaves of severely affected trees, however, may be mottled throughout the tree. Frequently the greater portion of the leaves borne by the shoots of a given growth cycle may be mottled, while those immediately preceding and following this cycle may be entirely normal in appearance. It is interesting, therefore, to compare the composition of normal and mottled leaves from the same tree.

With this end in view, samples of normal-appearing leaves were collected from the same trees from which some of the previously discussed samples of mottled leaves were drawn and on the same days. The analyses are reported in Tables XIV and XV.

The data are concordant with the previously reported analyses of normal leaves (Tables I and II). The results suggest that the leaves of different cycles of growth are mutually independent in composition and that the peculiarities in the composition of mottled leaves are not due to any special peculiarity of the tree upon which they have grown. A leaf of normal appearance borne by an orange tree the major portion of whose foliage is severely mottled, as were some of these samples, has approximately the same composition as any other normal orange leaf.

Some study has also been devoted to citrus trees affected by chlorosis[1] and injured by alkali, the results of which will be presented elsewhere.

The composition of albino and etiolated plants is of interest in this connection. Church (*4*, *5*) analyzed the normally green and albino portions of the maple (*Acer negundo*), holly (*Ilex aquifolium*), ivy (*Hedera helix*), and several other species. He found that the albino portions uniformly contained greater amounts of water than the green portions. The ash of the former contained greater amounts of potash and phosphoric acid and lesser amounts of lime than the latter, while the content of iron was approximately the same.

[1] Chlorosis of citrus, as it occurs in California, is distinguishable from mottle-leaf by a general fading of the chlorophyl over the entire mesophyl tissue, while mottle-leaf, as the name implies, denotes the lack of chlorophyl in spots between the veins.

TABLE XII.—*Composition of mottled lemon and grapefruit leaves*

LEMON

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Riverside, plot G	Nov. 4, 1917	2.33	0.26	26.94	3.10	11.64	1.56	4.82	5.02	0.27
Arlington	Dec. 10, 1917	1.77	.09	25.15	2.39	15.00	.21	5.33	4.50	.36
Average		2.04	.17	26.04	2.74	13.32	.88	5.07	4.76	.31

GRAPEFRUIT

Arlington	Dec. 10, 1917	1.72	0.08	24.28	2.57	15.31	0.29	5.63	3.95	0.29

TABLE XIII.—*Composition of mottled lemon and grapefruit leaves*

LEMON

Locality.	Date collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
		Per cent.	*Per cent.*										
Riverside, plot G	Nov. 4, 1917	67.82	13.99	0.32	0.037	3.77	0.43	1.63	0.22	0.25	0.34	2.72	0.04
Arlington	Dec. 10, 1917	65.95	13.85	.24	.013	3.84	.33	2.08	.03	.26	.36	2.40	.05
Average		66.88	13.92	.28	.025	3.80	.38	1.85	.12	.25	.35	2.56	.04

GRAPEFRUIT

Arlington	Dec. 10, 1917	64.83	16.09	0.28	0.013	3.91	0.41	2.46	0.05	0.32	0.33	2.86	0.05

TABLE XIV.—*Composition of normal orange leaves from trees bearing many mottled leaves*

Locality.	Date collected.	Constituents of ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
NAVEL										
Riverside, plot A	Dec. 9, 1916	2.88	0.16	30.77	1.46	7.49	0.98	2.44	3.94	0.36
Riverside, plot H	do	2.34	.15	31.71	2.07	5.94	.96	2.49	2.89	.57
Average		2.61	.15	31.24	1.76	6.71	.97	2.46	3.41	.46
VALENCIA										
Riverside, plot A	June 13, 1916	(a)	(a)	31.77	1.56	4.72	0.55	1.97	2.79	0.36
Riverside, plot H	do	(a)	(a)	33.76	1.68	3.01	.56	1.77	2.38	.17
Riverside, plot U	do	(a)	(a)	32.77	1.73	4.35	.52	1.35	2.50	.29
Average				32.77	1.66	4.06	.54	1.70	2.56	.27

[a] Not reported; results abnormally high because samples were contaminated with particles of dust.

TABLE XV.—*Composition of normal orange leaves from trees bearing many mottled leaves*

Locality.	Date collected.	Water.	Ash.	Constituents of ash, expressed as percentage of dry matter.									
				SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
		Per cent.	*Per cent.*										
NAVEL													
Riverside, plot A	Dec. 9, 1916	58.52	16.44	0.47	0.026	5.06	0.24	1.23	0.16	0.13	0.37	2.52	0.06
Riverside, plot H	do	55.59	17.52	.41	.027	5.56	.36	1.04	.17	.15	.37	2.60	.10
Average		57.05	16.98	.44	.026	5.31	.30	1.13	.16	.14	.37	2.56	.08
VALENCIA													
Riverside, plot A	June 13, 1916	57.63	17.32	(a)	(a)	5.50	0.27	0.82	0.09	0.12	0.29	2.23	0.06
Riverside, plot H	do	55.71	20.23	(a)	(a)	6.83	.36	.63	.11	.15	.34	2.35	.03
Riverside, plot U	do	58.33	18.05	(a)	(a)	5.91	.31	.78	.09	.14	.22	1.92	.05
Average		57.22	18.53			6.08	.31	.74	.10	.14	.28	2.17	.05

[a] Not reported.

Palladin (*14*) also found that the composition of the normal green and etiolated specimens of *Vicia faba*, the latter having been grown in the absence of light, differed in composition in the same general way as the normal and albino plants reported by Church. Weber (*16*) studied the effects of different parts of the spectrum on the composition of plants and found similar effects. Jensen (7) has recorded similar observations on the leaves of the privet plant, *Ligustrum aurea*.

While the fundamental cause of vegetable albinism is not known, the fact that light of certain wave lengths is essential to the formation of chlorophyl is well known; but in mottled citrus leaves the deficiency of chlorophyl certainly can not be caused by an insufficiency of light.

The fact that the composition of albino and etiolated plants differs from that of normal specimens in the same general way as is the case with mottled and normal citrus leaves shows that different causes may bring about similar effects in different species of plants. This fact also suggests at once that the composition of a plant may not afford a safe basis for forming a judgment as to the cause of a particular phenomenon. A satisfactory elucidation of these questions is not possible at present owing, in part at least, to the lack of definite knowledge concerning the fundamental principles underlying the growth processes of plants. The formation of chlorophyl is undoubtedly the result of a number of interdependent factors, and it is highly probable that either the absence or the inhibition of any one of these factors may prevent the formation of chlorophyl or ultimately lead to its decomposition.

COMPOSITION OF THE SAP OF ORANGE LEAVES

Some study has also been devoted to the sap of orange leaves. The sap was obtained by first subjecting the leaves to a temperature a few degrees centigrade below zero for a period of several hours Immediately after the leaves were removed from the freezing chamber they were quickly ground to a pulp with an ordinary meat grinder. The juice was then pressed from the pulp by the use of a hand-screw press. A portion of the juice was filtered through folded filter paper, and its specific gravity was determined by the pycnometer. Partial analysis was made on weighed portions of the juice by first evaporating to dryness and then using the methods previously described. Special investigations were also made on unfiltered portions of the sap as described below.

Mature normal leaves, collected from healthy navel orange trees on May 29, 1918, were first studied. A sample of 861 gm. of leaves yielded approximately 150 cc. of sap. Partial analysis gave the following results:

Specific gravity.	Ca.	K.	P.
	Per cent.	*Per cent.*	*Per cent.*
1. 08	1. 07	0. 54	0. 236

These data show that the expressed sap of mature orange leaves is comparatively rich in solids, calcium, and potassium, but the ratio of calcium to potassium in the sap is widely different from the ratio of the total amounts of these elements in the leaf. (Table II.)

On June 5, 1918, three sets of samples of Valencia orange leaves were collected. One of these was composed of normal leaves about 6 weeks of age; another sample obtained from the same trees consisted of healthy mature leaves; whereas the third sample was chosen to represent severely mottled leaves of the previous year's growth. Each of the samples was divided into three parts, one of which was used to study the sap, another to determine the water-soluble constituents, and the third for total analysis.

The sap was pressed out after freezing as described above. The water-soluble constituents were extracted by first grinding 100 gm. of the fresh leaves in a meat grinder, shaking with 1,000 cc. distilled water for one hour, and filtering through filter paper. Total acidity was determined by titration with *N*/10 sodium hydroxid, using phenolphthalein as indicator. It was necessary to dilute the sap considerably because of its dark color, and a high degree of accuracy is not claimed for the results. They are rather approximations. The acidity is expressed for convenience as anhydrous citric acid.[1] The results are presented in Tables XVI, XVII, and XVIII.

TABLE XVI.—*Composition of Valencia orange leaves at the age of 6 weeks*

	Specific gravity.	Ash.	Ca.	K.	P.	N.	Acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Sap	1.065	3.17	0.67	0.72	0.045	.....	.....
Water extract [a]	1.005	.....	1.57	1.69	.13	.....	1.64
Total leaf [a]	.....	13.23	3.56	1.99	.21	2.45	.....

[a] Expressed in terms of dry matter.

TABLE XVII.—*Composition of normal mature Valencia leaves*

	Specific gravity.	Ash.	Ca.	K.	P.	N.	Acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Sap	1.097	4.32	1.41	0.42	0.035	.....	.....
Water extract [a]	1.008	.....	2.85	.64	.063	.....	1.15
Total leaf [a]	.....	17.56	5.78	.94	.13	1.92	.....

[a] Expressed in terms of dry matter.

[1] The nature of the acid constituents of the leaves has not been investigated sufficiently to justify a definite statement as to their identity.

The results show that the sap of Valencia orange leaves at the age of 6 weeks contains smaller amounts of dissolved solids and total ash material than mature leaves. The calcium content increases more than twofold, and the potassium and phosphorus content decreases in passing to maturity. On the other hand, the sap of mottled leaves has a higher specific gravity and a higher ash content than that of mature normal leaves. The calcium content, however, is considerably less, while the potassium and phosphorus content is much higher.

It is evident from these data, therefore, that the sap of mottled Valencia orange leaves is materially different from that of normal leaves, either when they are 6 weeks of age or mature.

The water-soluble constituents were found to diverge in the same general direction as the sap. It is interesting to note that a very high percentage of the potassium, phosphorus, and calcium of orange leaves is soluble in water.

Samples of fully mature normal leaves and of severely mottled leaves of the previous year's growth were collected from Navel orange trees of the fertilizer plots at Riverside in August, 1918. The sap was expressed and used for more complete chemical study. (Tables XIX and XX.)

TABLE XVIII.—*Composition of mottled Valencia leaves*

	Specific gravity.	Ash.	Ca.	K.	P.	N.	Acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Sap	1.118	4.85	1.13	0.91	0.111	.....	.....
Water extract [a]	1.009	.....	2.85	1.64	.180	.....	2.75
Total leaf [a]	.....	15.06	4.05	1.98	.243	3.00	.....

[a] Expressed in terms of dry matter.

The results are fairly concordant with those reported above for Valencia leaves. It is again shown that the composition of the sap of mottled orange leaves differs widely from that of normal leaves. The data also show that the ash of the sap of each sample contained considerably smaller percentages of calcium and higher percentages of iron than those reported above for the ash of the leaf as a whole, while the percentages of the other constituents are not materially different from those of the entire leaf. The calcium content of the sap of Navel orange leaves appears to be lower than that of Valencia leaves. (Compare Tables XVII and XX.)

Upon studying the preceding data, it seems difficult to escape the conclusion that there must be some important physiological significance attached to the fact that the sap of mottled orange leaves contains only about one-half as much calcium and approximately twice as much potassium and nitrogen and three times as much phosphorus as normal leaves.

TABLE XIX.—*Composition of the sap of mature normal and mottled Navel orange leaves*

Condition of leaves.	Specific gravity.	Constituents of sap, expressed as percentage of its ash.								
		SiO_2.	Fe.	Ca.	Mg.	K.	Na.	PO_4.	SO_4.	Cl.
Normal	1.068	0.43	0.37	19.71	2.89	8.05	1.49	2.30	5.58	3.13
Mottled	1.074	.46	.36	9.88	2.30	17.32	.43	6.27	4.01	.82

TABLE XX.—*Composition of the sap of normal and mottled Navel orange leaves*

Condition of leaves.	Ash.	SiO_2.	Fe.	Ca.	Mg.	K.	Na.	P.	S.	N.	Cl.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Normal	4.51	0.19	0.017	0.89	0.13	0.36	0.07	0.034	0.11	0.54	0.14
Mottled	4.89	.023	.018	.48	.10	.84	.02	.100	.09	.95	.04

The hydrogen-ion concentration of the sap was also determined by the use of the hydrogen electrode. Mature normal-leaf sap was found to give a P_H value of 5.816 and mottled-leaf sap a value of 5.647, which implies hydrogen-ion concentrations of 0.153×10^{-5} and 0.226×10^{-5}, respectively. These determinations are probably within the range of variation of different samples of the same leaves.

After the determination of the hydrogen-ion concentration, total acidity was determined by titration, using the hydrogen electrode to determine the end point. It was found that 10 cc. of the normal sap required 3 cc. *N/10* alkali and the mottled-leaf sap 7.05 cc. In other words, the actual acidity (hydrogen-ion concentration) of mottled-leaf sap is approximately the same as that of normal leaves, but the latter sap is more nearly saturated with base. It is probable that in each case the ionization of the acids is held at approximately the same level by the buffers present.

Samples of normal Navel orange leaves approximately one week of age, fully mature leaves, and severely mottled leaves of the previous year's growth were collected in April, 1919. The sap was expressed, and the hydrogen-ion concentration and total acidity were determined by the hydrogen electrode. Freezing-point depressions were also determined in portions of the unfiltered sap. The acidity is expressed in cubic centimeters of *N/10* sodium hydroxid required to neutralize 10 cc. of the sap.

TABLE XXI.—*Acidity and freezing-point depression of orange-leaf sap*

Condition of leaves.	P_H.	Hydrogen-ion concentration.	Total acidity.	Freezing-point depression.
			Cc.	°C.
Normal, 1 week old	6.069	0.852×10^{-6}	1.80	1.258
Normal, mature	5.664	$.217 \times 10^{-5}$	3.80	1.588
Mottled	5.647	$.226 \times 10^{-5}$	7.00	1.734
Mottled	5.630	$.235 \times 10^{-5}$	8.25	.....

These data show that the actual acidity (hydrogen-ion concentration) of mature orange-leaf sap is approximately two and one-half times as great as that of leaves at the age of 1 week; but again it is shown that the acidity of mottled leaves is approximately the same as that of normal leaves. The capacity to neutralize base—that is, total acidity—however, was fully twice as great in mature leaves as in those 1 week of age, while the mottled-leaf sap neutralized about twice as much base as the normal mature leaf sap.

The freezing-point depressions show that while the normal mature-leaf sap is more concentrated than that of young leaves the sap of mottled leaves is more concentrated than either.

The results of the preceding investigation on the sap of orange leaves are very suggestive. They are in harmony with the preceding ash

analyses in that they indicate that the composition changes materially as growth proceeds and that the composition of mottled leaves differs from that of normal leaves.

It is interesting to note that the total water content of mottled and normal mature leaves is roughly correlated with the concentration of the sap, but this correlation does not hold when immature leaves are compared with mature leaves.

GENERAL DISCUSSION

It has been shown that the composition of orange leaves changes rapidly as growth takes place. The relationships between the several constituents drawn from the soil undergo important alterations. The percentages of potassium and phosphorus, when expressed on the basis of either the ash or the dry matter, decline rapidly during the early part of the growth cycle and continue to decline, although at reduced rates, during the latter part of the growth period. The percentages of nitrogen in the dry matter also decrease as growth proceeds. The percentage of calcium, on the other hand, increases rapidly at first, and later more slowly. The concentration of iron is greatest in very young leaves, but later its concentration decreases slowly, while no very pronounced changes take place in the percentages of the other constituents. The concentration of the different constituents probably remains practically constant throughout the period of normal maturity.

As the leaves approach senility just preceding the time of normal dropping, notable amounts of potassium and nitrogen are translocated back into the stem or other portions of the tree. A part of the phosphorus also appears to leave the leaf sometime preceding the period of normal maturity. In contrast to certain cereals, the absolute content of magnesium does not decrease as maturity approaches.

It has been shown that a given orange leaf normally contains the maximum amounts of potassium, phosphorus, and nitrogen by the time it is approximately 6 weeks of age. It is interesting that the leaf also reaches its maximum size about the same time. On the other hand, the absolute content of calcium continues to increase until full maturity is reached.

Mature orange leaves are extremely rich in certain nutrients. The content of carbonated ash ranges from 14 to 18 per cent of the dry matter, and the nitrogen content is usually above 2 per cent. The most pronounced characteristic of the orange leaf, however, is found in its highly calcareous nature. When the leaf is mature, the dry matter contains from 5 to 6 per cent of calcium.

Lemon and grapefruit leaves are similar in composition to orange leaves.

The composition of mottled citrus leaves is widely different from that of normal leaves. The difference lies mainly in the smaller calcium content, on the one hand, and the greater content of potassium and

phosphorus, on the other. Usually the nitrogen content of mottled leaves is also abnormally high. The composition of mottled orange leaves resembles that of immature leaves, although the percentages of ash and nitrogen in the former are materially greater than in the latter.

It has been shown that the absolute amounts of potassium and phosphorus contained in mottled orange leaves are fully as great as ordinarily occur in normal leaves that are two or three times as large, while the calcium content is not more than one-third that occurring in average normal leaves.

The sap of normal orange leaves becomes increasingly concentrated and acidic as growth proceeds. When mature it is especially rich in calcium and contains fully twice as much of this element as of potassium.

The abnormalities of mottled leaves noted above also occur in the sap and among the water-soluble constituents. The sap of mottled leaves contains subnormal amounts of calcium and fully twice as high concentrations of potassium and phosphorus as mature normal leaves. The hydrogen-ion concentration of mottled leaves is not materially different from that of normal leaves, but the sap is less nearly saturated with base. In other words, abnormally large amounts of unionized acids occur in mottled-leaf sap.

Limited study of portions of citrus trees other than the leaves indicates that the composition of the leaf spurs of severely mottled trees varies from the normal in much the same way as the leaves. The composition of the older wood, however, is more nearly normal. On the other hand, both the large roots and small rootlets of severely mottled trees appear to contain considerably less potassium and phosphorus than normal roots, while the calcium content is approximately normal.

Should more extended study confirm these latter observations, it would seem that the excessive proportions of potassium and phosphorus occurring in mottled leaves may have been drawn, in part at least, from the supply normally stored in the roots.

The results of these investigations suggest that mottled citrus trees are deficient in calcium, but the cause of the subnormal content of calcium can not be definitely stated.

While we recognize that growing plants have the power, through selective absorption, of regulating their composition to a marked degree, and that a given variation in the composition of a plant does not necessarily reflect a corresponding deficiency in the nutrient medium, the above data suggest that the abnormalities in the composition of different parts of mottled citrus trees may be due, in part at least, to the inability of the tree to satisfy its normal calcium requirements at critical periods.

It is well known that manure and other forms of decaying organic matter exert an ameliorating effect on mottle-leaf. It is interesting in this connection that the concentration of soluble calcium in the soil

becomes materially increased as a result of the decomposition of such materials (*8*). On the other hand, the occurrence of heavily compacted layers of soil (plowsole) around the roots, especially when present immediately below the depth of cultivation, and of soils of low organic content (*3*) and low natural solubility afford conditions that are conducive to mottle-leaf. Where such conditions occur, it is possible that the supplies of those nutrients which are normally absorbed at relatively high rates may become inadequate. The nature and extent of the root system of citrus trees must also be considered in this connection. It is interesting that the absorbing roots of citrus trees are not provided with the usual root hairs. Consequently, they may possess less absorbing surface than is afforded by other plants that normally absorb relatively large amounts of nutrients. These and other related questions will be more fully discussed elsewhere.

The fact that mottle-leaf sometimes appears on trees that have been injured by alkali suggests the possibility that alterations in permeability occasioned by the presence of excessive concentrations of salts, or possibly toxic substances of other kinds in the soil moisture, may prevent the roots from taking up normal amounts of calcium.[1]

If we may judge from the composition of normal leaves, the calcium requirements during the period when mottle-leaf develops most pronouncedly are extremely heavy. The leaves at that stage normally absorb calcium at a high rate.

Just why subnormal concentrations of calcium accompanied by supernormal concentrations of potassium and phosphorus in the leaves should afford conditions that tend to limit chlorophyl production is not known, if indeed further investigations prove that such is the case. There may, of course, be no causal relationship between these facts, but rather each may be the result of causes not yet suggested.

It is recognized that calcium is not a normal constituent of chlorophyl. In addition, while iron is essential to the formation of chlorophyl yet does not enter into its final composition, we are not aware that a similar relationship exists between calcium and chlorophyl formation. Consequently, even though further study should prove that mottle-leaf can be produced as a result of an inadequate supply of available calcium, it is probable that the lack of chlorophyl and its disappearance from the localized areas of the leaves would be found to be indirect rather than direct effects of a shortage of calcium. In any event, whether the shortage of calcium or some other factor conditions the deficiency of chlorophyl, photosynthesis is doubtless reduced by the lack of chlorophyl.

With an adequate supply of nitrogen, phosphorus, and potassium present in the soil moisture, osmosis might bring about the absorption of

[1] As is well known, the occurrence of mottle-leaf is sometimes correlated with the species of root stock, but this phase of the subject has not been systematically investigated in California. Mr. H. Atherton Lee has called the writer's attention to his studies on this phase of mottle-leaf in the Philippine Islands.

greater or lesser amounts of them, despite the deficiency of chlorophyl in the leaves; but the reasons why excessive amounts of these elements accumulate in mottled citrus leaves are not clear. It seems probable that some physico-chemical principle not elucidated by the preceding data must be fundamentally involved.

Before any explanation of mottle-leaf can be safely accepted, it is necessary to show that the disease can be produced experimentally, and that too under conditions admitting of scientific analysis. Additional studies already projected may throw further light on this subject.

Whatever may ultimately be found to be the primary cause of mottle-leaf, the preceding investigations strongly suggest that the leaves are not suffering from inadequate supplies of potassium, phosphorus, or nitrogen. We have also found little, if any, indication of a deficiency of iron.

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CONTROL OF FLUKE DISEASES BY DESTRUCTION OF THE INTERMEDIATE HOST[1]

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Flukes have long been known as causative agents of disease in animals, especially sheep; in fact the loss resulting from their ravages in some sheep-raising countries can be estimated in millions of dollars annually. Within comparatively recent years flukes have been discovered to play an important rôle in some countries in the production of human disease. At present human fluke infections are known to be more or less prevalent in nearly all tropical and subtropical countries and in some countries of temperate climate. The blood flukes, Schistosoma, occur in the oriental countries and throughout most of Africa and tropical America. Human liver flukes, Clonorchis, and the lung flukes, Paragonimus, are primarily diseases of the Orient, but epidemic cases have been reported from other countries. The various species of intestinal flukes which are habitual or accidental human parasites occur in both Asia and Africa and probably in other tropical countries, but these are of minor importance.

The important relation of fluke infections to the public health in endemic countries is not generally realized. In Egypt, for instance, over half the population is said to suffer from schistosomiasis, and in an examination of 54 boys in the village of El Marg, near Cairo, Leiper (*10*)[2] found 49 to be infected. Cawston (*3*) states that in some districts in South Africa 80 per cent of the school boys and 10 per cent of the girls are infected and that Schistosoma infections seriously retard both the physical and mental development of the school children. Troops operating in endemic regions are much affected by the disease unless stringent preventive measures are taken. The British army suffered severely in the Boer war, and in 1914 the British Government was still under heavy expense for pensions for soldiers invalided by schistosomiasis. Laning, of the United States Navy, states that it is not uncommon for large proportions of the crews of patrol gunboats operating on the Yangtze River to be completely disabled by *Schistosoma japonicum* infections. Nakagawa (*13*) states that lung flukes are harbored by as high as 50 per cent of the population in some districts in Formosa, and in parts of Japan the infection is hardly less prevalent. Clonorchis, a human liver

[1] Contribution from the Zoological Laboratory, Oregon Agricultural Experiment Station, Corvallis, Oreg., and from the Biological Laboratory, Rice Institute, Houston, Tex.

[2] Reference is made by number (italic) to "Literature cited," p. 208.

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fluke, is even more prevalent in Japan and is said by Kobayashi (*9*) to affect as many as 60 per cent of the inhabitants of some endemic areas. Like malaria and hookworm disease, fluke diseases are comparatively seldom fatal in themselves but are particularly injurious in causing loss of efficiency, reduced vitality, and lowered resistance to other diseases. The long duration and relative incurability of fluke infections are a very serious factor. In this respect fluke infections are far more to be feared than are infections with intestinal parasites, most of which are relatively easy to expel. Of the numerous drugs which have been tried in the treatment of extra-intestinal fluke infections, only tartar emetic, recently shown by Christopherson (*4, 5, 6, 7*) to be more or less specific in its action on Schistosoma, gives promise of being of any great value. No unquestionably effective remedy for lung or liver flukes has been found, and even the use of tartar emetic for schistosomiasis is far from satisfactory, since the serious symptoms of the disease are caused by the eggs of the worms deposited in the tissues and often continue to exist long after the worms are dead.

With regard to fluke diseases of domestic animals the situation is no less serious. The common liver fluke of sheep and cattle, *Fasciola hepatica*, is found almost all over the world in temperate climates, being prevalent wherever these domestic animals are grazed on wet or marshy pastures. In the British Isles, France, Germany, and other parts of Europe and in some parts of the United States, notably western Oregon and Washington and the humid districts of Texas, Louisiana, Florida, and other southern States, the losses from flukes in sheep, cattle, and goats amounts to millions of dollars annually, on account of loss of vitality among the animals, depreciation in quantity and quality of meat, and the loss of the infested livers themselves. In the Tropics Fasciola is largely replaced by other flukes—for example, the intestinal Amphistoma and Gastrodiscus, and various blood flukes, Schistosoma. As with human flukes, the extra-intestinal flukes of animals can not be reached readily by drugs, and as pointed out by Ransom and Hall (*16*) there is still much doubt about the efficacy of drugs which have been recommended for use against them, though there is room for much more experimentation.

On account of the difficulty encountered in treating or curing fluke diseases, preventive measures loom up with even greater importance than they do in dealing with hookworm or other intestinal parasites. The working out of preventive measures based on scientific knowledge has only recently become possible, for, although the life history and mode of infection of the common liver fluke, *Fasciola hepatica*, of sheep and cattle have been well known for a number of decades, such knowledge of human flukes has been acquired only in the past three or four years. Leiper's work on Schistosoma in Egypt in 1915-16 (*10*), Kobayashi's work on Clonorchis in Japan in 1915 (*9*), and Nakagawa's work on

Paragonimus in Formosa in 1916 (*13*) have given a definite basis for preventive measures against all these parasites of man and of the related parasites of domestic animals.

In every case of fluke infection of man or domestic animals in which the life cycle of the parasite has been worked out it has been shown that fresh-water snails act as necessary intermediate hosts. It appears, therefore, that if some efficient and practical method of destroying the snails could be found, this would furnish a logical point of attack in the control of all fluke diseases. Other preventive measures are, of course, valuable also and could be used as supplementary measures—for example, the impounding of water before use for drinking or bathing as a preventive against Schistosoma infections, the discouragement of the habit of eating improperly cooked meat of crabs in the case of Paragonimus and of fish in the case of Clonorchis, and care in the disposal of feces and urine in all cases. The last, exclusive of individual mechanical protection against infection, is the only preventive measure that can be adopted against hookworm and many other intestinal parasites. To accomplish this in some warm countries where there has never existed anything approaching sanitation and where the very idea of sanitation is so strange and foreign to the habits of life and thought of the natives is well nigh impossible. The fact, therefore, that fluke infections may possibly be controlled by attack upon an intermediate host instead of by reliance upon the enforcement of sanitary regulations makes the ultimate eradication of these infections, in spite of their relative incurability, a matter of brighter prospect than is the case with many other verminous parasites.

Already a number of suggestions for the destruction of the snails which act as intermediate hosts of flukes have been made. Thomas (*18*) advised the extensive scattering of salt on pastures where sheep were known to become infected by flukes, and he commented on the absence of fluke infestations among sheep grazing on salt marshes. The effect of the salt, of course, was to destroy the snail, Limnaea, which acts as the intermediate host. Leiper (*10*) suggested the eradication of the disease in agricultural districts in Egypt by the intermittent flow of water in the irrigation ditches, the water being turned off for 15-day periods, thus drying up the ditches and destroying the snails by desiccation. Such a procedure is, of course, very limited in its application, and in view of the remarkable resistance which many snails have to drouth it is doubtful whether all the implicated species could be killed by this method even if it were feasible. Leiper suggested that ammonium sulphate be applied to pools which were inhabited by the intermediate hosts of Schistosoma. Lime has been recommended by a number of writers, particularly Japanese, as the cheapest and best method of destroying snails. One Japanese writer, Ando (*1*) states that 1 per cent lime water killed 6 of 10 snails in seven hours, and a 1 per cent solution of copper sulphate would kill them in six hours. It is obvious that none of the above methods of

exterminating snails would be practical on a large scale, either on account of the prohibitive cost or on account of the excessive amounts of the material used and consequent injury to the water for drinking, bathing, or irrigation purposes.

In the hope of finding some effective means of destroying disease-carrying fresh-water snails a series of experiments was undertaken by the writer. The original purpose of the investigation was to find a solution to the liver fluke problem among sheep and cattle raisers in the Willamette Valley of Oregon, but it was realized that if a means of controlling all fresh-water snails could be found, the results would be of infinitely greater value than the solution of the local problem, and the experiments were carried on with this in mind.[1]

It was obvious that any chemical which could be used on a large scale for the destruction of snails in ponds, marshes, or streams must not be toxic to man or domestic animals in the dilutions used and must not be expensive. An attempt, therefore, was made to find a cheap chemical substance, readily soluble in water, which would be destructive to snails in relatively weak solutions and which would not render water either injurious or unpalatable for man or domestic animals.

The chemicals which were selected for preliminary experiments, the dilutions which were made, and the results obtained are shown in Table I. The snails, *Limnaea (Galba) bulimoides*,[2] were immersed in each solution, using chemically pure salts and tap water, Corvallis tap water being unusually clear, pure, and soft. The sign − indicates no evident effect, ± slight noticeable effect in behavior, + distinct illness without complete prostration, + + complete prostration, and ⊕ death. It was found later that snails which were apparently dead would sometimes revive if placed in fresh, aerated water; therefore the results shown in this table are not absolutely dependable. They do, however, demonstrate beyond question one striking thing—the fact that copper salts have an extremely toxic effect on these snails, even in such great dilutions as one part to a million of water. Mercuric bichlorid is the only other salt experimented with which approaches the salts of copper in its toxicity to snails, but since it is evidently not so effective as copper, is more toxic to higher animals, and is more expensive, no further experiments with it were carried out.

The salts of copper being evidently the most promising substance with which to attack aquatic snails all subsequent work was concentrated on them. Experiments with various copper salts ($CuCl_2$, $CuSO_4$, $Cu[NO_3]_2$) were tried, and it was found that with equivalent concentrations of the Cu_{++} ion their toxicity was approximately the same. Copper sulphate,

[1] The writer has been unable to get access to the following paper: GERMAIN, L. DE L'EFFET DES POISONS MINÉRAUX SUR QUELQUES MOLLUSQUES TERRESTRES ET FLUVIATILES DE FRANCE. *In* Bul. Soc. Amis Sci. Nat. Rouen, s. 4, ann. 34, 1898, sem. 1, p. 71–78. 1899.

[2] Snails specifically named in this paper were kindly identified by Dr. H. A. Pilsbry, Dr. F. C. Baker, or Mr. Bryant Walker.

being the cheapest copper salt, was therefore selected for further experimentation.

Chemical.	Dilution.	1 hour.	4 hours.	8 hours.	24 hours.
As_2O_3	1 to 1,000,000	−	8 ±[a] 2 −	±	−
$Ba(NO_3)_2$	1 to 100,000	−	−	−	−
$CaOCl_2$	1.3 available chlorin per 1,000,000.	−	−	−	−
$CaOCl_2$	2.6 available chlorin per 1,000,000.	−	−	−	−
$Ca(OH)_2$	1 to 10,000	−	−	−	−
$CuCl_2$	1 to 100,000	++	++	⊕	⊕
$CuSO_4$	1 to 100,000	++	++	⊕	⊕
$CuSO_4$	1 to 1,000,000	+	++	++	⊕
$HgCl_2$	1 to 1,000,000	±	++	++	7⊕ 3+
NaCl	1 to 1,000	−	−	−	−
NaCN	1 to 100,000	−	−	−	−
NaCN	1 to 1,000,000	−	−	−	−
$(NH_4)_2SO_4$	1 to 100,000	−	−	−	−
$(NH_4)_2SO_4$	1 to 1,000,000	−	−	−	−
$Pb(CH_2COOH)_2$	1 to 100,000	−	±	6+ 4−	2++ 6+ 2−
$Pb(CH_2COOH)_2$	1 to 1,000,000	−	−	±	±
$ZnCl_2$	1 to 1,000,000	−	−	−	−
$ZnSO_4$	1 to 1,000,000	−	−	−	±

[a] Figures beside symbols indicate number of snails out of the 10 used in the experiment.

The effect of copper salts on various kinds of organisms is extremely variable. Their highly toxic effect on algae, first demonstrated by Moore and Kellerman (*11*), is well known, and copper sulphate is extensively and successfully used in eliminating algae from ponds and reservoirs. Copper sulphate is effective against some algae in dilutions up to 1 part in 25,000,000 or more parts of water but is commonly used in the proportion of 1 part to from 1,000,000 to 3,000,000 parts of water. Its bactericidal action is less marked and varies greatly with temperature. At 20° C., in water relatively free from organic matter, all pathogenic bacteria are destroyed in 24 hours at a dilution of 1 part to 400,000 parts of water. Peters (*14*) showed that the concentration necessary to kill instantly certain protozoa was 12 to 60 × 10^{-3} gram molecular parts per cubic centimeter of water (about 3 to 15 parts per million). The toxic effect of copper on fungi is as striking as its effect on algae and is taken advantage of commercially in the use of Bordeaux mixture for spraying trees and vines.

Curiously enough the effect of copper salts on both higher plants and higher animals is in general far less toxic than it is on lower animals and plants. In dilute solutions copper sulphate has a stimulating action on the growth of many higher plants, having been tested particularly on various grains. In the animal series, copper salts are usually

harmless in the dilutions which are lethal to the single-celled organisms. Copper is, in fact, a normal constituent of their tissues and replaces iron in the blood of some invertebrates. Experiments by the writer, as well as by others, show that copper, 1 part per million, is not injurious, at least within 48 hours, to annelids, crustaceans, or aquatic insect larvae. Of vertebrate animals, fish are highly susceptible, various species being affected by 1 part of copper sulphate in from 500,000 to 10,000,000 parts of water. Amphibians are immune to dilutions of 1 to 1,000,000. Contrary to popular opinion, copper is not highly toxic to mammals and can, in fact, be taken by the mouth in considerable quantities without injury. Five to 10 gr. (0.32 to 0.64 gm.) can be taken as an emetic. Horses and cattle can take 3.9 to 7.7 gm. and sheep 1.3 to 2.6 gm. It is evident, therefore, that copper salts in high dilution have a selective effect on various organisms, being particularly destructive to single-celled organisms, certain molluscs, and fishes. For destroying aquatic snails, therefore, copper sulphate can be used in perfect safety so far as any possibility of injury to man or domestic animals from drinking or bathing is concerned, without injuring the water for irrigation purposes, and without destroying other higher organisms, except certain species of fish.

After it was found that very dilute solutions of copper salts are specifically toxic to *Limnaea (Galba) bulimoides*, experiments were carried out to determine their effect on other species of snails and also to ascertain as accurately as possible the effect of varying concentrations of the salts. In all of these experiments only chemically pure copper sulphate was used. Preliminary experiments showed that there was no appreciable difference in effect whether distilled water or the local tap water was used in the experiments; therefore the tap water was used except for making up the stock 0.1 per cent and 0.01 per cent solutions. All the local species of snails of which sufficient numbers could be obtained were tried. It was not practicable to experiment with the species of snails which have actually been incriminated as the intermediate hosts of important flukes of man and domestic animals, but a greater variety of snails than those which have been incriminated were used, including representatives or close allies of all the incriminated families and in some cases genera.

Of the species used, *Planorbis callioglyptus* belongs to the family Planorbidæ, to which belong Bullinus, Planorbis, and Physopsis, intermediate hosts of *Schistosoma hæmatobium* and *S. mansoni;* Goniobasis, according to Pilsbry, is closely akin to Melania, intermediate host of Paragonimus, Metagonimus, and Clonorchis; Fluminicola belongs to the family Amnicolidæ in common with Blanfordia, intermediate host of *Schistosoma japonicum;* several species of Limnaea serve as intermediate hosts for *Fasciola hepatica.*

Some difficulty was encountered in correctly reading the effect produced on the snails, and all earlier experiments had to be discarded.

It was found that snails which were prostrate and would not respond to stimuli, and were therefore apparently dead, would frequently revive after being placed in fresh, aerated water for from 12 to 24 hours. The only criterion for death which was used, therefore, was failure to revive within 24 hours after being placed in fresh water.

Experiments, using 10 snails of a species in one liter of the solution at approximately 18° to 20° C., were made as follows:

DILUTION.	SPECIES TESTED.
1 to 100,000	*Goniobasis plicifera, Limnaea bulimoides, Physa occidentalis.*
1 to 500,000	*Fluminicola fusca, Goniobasis plicifera, Physa occidentalis.*
1 to 1,000,000	*Ancylus caurinus, Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, L. proxima rowelli, Physa nuttalli, P. occidentalis, Planorbis callioglyptus.*
1 to 1,500,000	*Fluminicola fusca, Goniobasis plicifera, Physa occidentalis.*
1 to 2,000,000	*Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, L. proxima rowelli, Physa occidentalis, P. nutalli, Planorbis callioglyptus.*
1 to 2,500,000	*Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, L. proxima rowelli, Physa occidentalis.*
1 to 3,000,000	*Fluminicola fusca, Goniobasis plicifera, Limnaea bulimoides, Physa occidentalis.*
1 to 4,000,000	*Physa occidentalis.*
1 to 5,000,000	*Fluminicola fusca, Goniobasis plicifera. Limnaea bulimoides, L. Proxima rowelli, Physa nuttalli, Physa occidentalis.*
1 to 10,000,000	*Limnaea bulimoides, Physa occidentalis.*

The results of these experiments may best be summarized as follows:

1. All species of snails experimented with, eight in all, belonging to six genera and as many families, are similar to each other in their susceptibility to copper sulphate. There is, in fact, much more individual variation shown than there is difference between species. Ancylus, Fluminicola, Limnaea, and Planorbis become prostrate a little more quickly than do Goniobasis and Physa. Goniobasis has a little more recuperative power than the other species after being placed in pure water.

2. All species die within 48 hours, many specimens sooner, in solutions of 1 to 500,000 and 1 to 1,000,000. Fluminicola, Limnaea, Physa, and Planorbis die within 48 hours in solutions of 1 to 1,500,000 and 1 to 2,000,000, but a few specimens of Goniobasis and one specimen of *Limnaea proxima rowelli* revived slightly after being placed in fresh water for 24 hours but died within 48 hours.

3. A 1 to 500,000 solution appeared to be no swifter in its action than was a solution of 1 to 2,000,000. In all dilutions between 1 to 500,000 and 1 to 2,000,000 some specimens revived after exposure for 24 hours, whereas after 48 hours none revived except as noted in the preceding paragraph.

4. Solutions ranging between 1 to 2,500,000 and 1 to 5,000,000 killed 50 per cent or more of the specimens, but not all, whereas all specimens revived after 48 hours in a 1 to 10,000,000 solution, although they became sick or prostrate while immersed in it.

5. In the 1 to 100,000 dilution, which was tried merely to ascertain whether this concentration would kill quickly, the snails became prostrate immediately upon being immersed and remained motionless, but they almost all revived after immersion for one hour.

The actual physiological effect of the copper salts on the snails has not been determined. Within a few minutes the snails immersed in a dilute copper-sulphate solution lie prostrate, being apparently unable to cling to the sides of the jar. A mucous albuminoid substance is exuded, and frequently feces, eggs, and even the penis, are extruded. It is highly probable that the poisoning effect is due at least in part to inactivation of enzyms necessary to life. Peters and Burres (*15*) showed that the concentrations of copper sulphate necessary to kill Paramoecium and Stentor were approximately the same as those necessary to inactivate their normal enzyms. It was thought that possibly there was a special tendency in snails to absorb copper, since this metal is an important constituent of the blood and is found only in minute traces in the normal environment. However, analyses of snails killed in copper-sulphate solutions, compared with normal snails, failed to show appreciably greater quantities of copper. Furthermore it was found that the snails succumbed as quickly in a few cubic centimeters of the solutions as they did in large quantities. Five specimens of *Limnaea bulimoides* were killed in 10 cc. of a 1 to 1,000,000 solution, yet the total amount of copper present was only about 0.0025 mgm., or 0.005 mgm. per snail. By analogy with *Helix pomatia*, which was shown by Dubois (*8*) to contain 6.11 mgm. of copper per 100 gr. of body weight, a specimen of Limnaea should normally contain several milligrams of copper. If the mode of action of the copper salts is by inactivation of enzyms, the similarity in effect of such varying dilutions as 1 to 500,000 and 1 to 2,000,000 is more readily explained.

The effect of a 1 to 1,000,000 copper-sulphate solution was also tried on the eggs of *Physa nuttalli* and of *Limnaea bulimoides*. Eggs in intact gelatinous masses were apparently uninjured by the copper solutions in 14 days, though the inclosed embryos seemed to grow more slowly than the controls.

There are a number of factors which influence the effect of copper sulphate on organisms in water, the most important being temperature, presence of algae, alkalinity, and organic matter in solution. As regards temperature, no extended experiments were carried out, but experiments with a 1 to 1,000,000 solution were carried out at temperatures of from 15° to 27° C., and the snails apparently succumbed as quickly at the lower as at the higher temperature. Water in which snails were to be destroyed would probably not fall below 15° C. in temperature. Alkalinity of water, to the extent normally found in natural ground waters, appears to have little effect on the action of the copper salts, although copper sulphate is precipitated as basic sulphates or carbonates in

alkaline solutions. The tap water at Rice Institute, which is strongly alkaline because of the presence of sodium carbonate, when used in a 1 to 1,000,000 solution of copper sulphate was apparently as effective as distilled water, even after standing for 24 hours to allow time for possible precipitation of the copper.

Since organic matter in solution rapidly precipitates copper, water containing considerable quantities of it should receive larger quantities of copper sulphate to make up for loss by precipitation. The concentration of copper sulphate necessary to destroy typhoid bacilli, according to Rettger and Endicott (*17*), was four times as great in water containing 0.01 per cent peptone as in distilled water and 40 times as great in the presence of 1 per cent peptone. Moore and Kellerman (*12*) advise an increase of 2 per cent in the concentration used to kill algae for each part per 100,000 of organic matter. It is probable that a similar increase in the amount of copper used against snails would be sufficient to counteract the effect of the organic matter.

The presence of algae in the water has a marked effect on the action of copper salts on snails, since the algae, which are killed by the salts, absorb them. Bado (*2*) has demonstrated considerable quantities of copper in the ash of algae which had been exposed to copper sulphate in dilute solution, and he states that it is absorbed at different rates by different species. In a preliminary experiment, the writer found that snails placed in one liter of a 1 to 1,000,000 solution of copper sulphate, together with a large handful of algae (Vaucheria and attached diatoms) although they showed symptoms for a few hours after immersion, subsequently revived and on the following day were as active as the controls. To test more accurately the effect of algae, a quantity of fresh green algae was rinsed and then squeezed like a sponge until water was no longer expelled by moderate pressure. Quantities of this weighing 0.25, 0.5, 1, 2, 3, 4, and 5 gm. were placed in liters of a 1 to 1,000,000 copper-sulphate solution, and snails (*Physa occidentalis*) were placed in each. The snails in the jars containing up to 1 gm. of the wet algae (1 gm. = 150 mgm. dry weight) died as quickly as did the controls in a simple copper-sulphate solution. Those in jars containing 2, 3, and 4 gm., although prostrate within 24 hours, still responded weakly to stimuli at the end of 48 hours but did not revive when placed in fresh water. One-third of the snails in the jar with 5 gm. of algae partially revived in the solution. A second experiment, similarly conducted, but with the use of Spirogyra, one of the algae most susceptible to copper salts, was tried. In this experiment only 1, 2, and 3 gm. quantities were used. The snails with 1 gm. of Spirogyra did not die within 48 hours but failed to revive in fresh water and died within 48 hours after being refreshed. Of those with 2 gm. 50 per cent revived after being refreshed, whereas of those with 3 gm. all revived.

As shown in the preliminary experiments with various chemicals, chlorinated lime up to double the amount used for sterilizing drinking water does not affect snails at all. It was found, furthermore, that the presence of chlorinated lime in the proportion of 1 to 250,000 (about 1.3 parts available chlorin per million) had an inhibiting effect on the action of copper sulphate on snails to such an extent that some specimens did not even become prostrate in the solution. The mode of interaction of the copper sulphate and chlorinated lime was not investigated, but it is possible either that the liberated oxygen from the chlorinated lime may counteract the effect of the copper sulphate on the enzyms, or that a chemical reaction takes place which precipitates the copper. If the latter is true it might be feasible to remove copper sulphate from solution in water by the use of chlorinated lime, in case this should for any reason be desirable after using it in destroying algae, snails, or other organisms.

A number of practical field experiments were carried out to demonstrate the effectiveness of copper-sulphate treatment for snails in actual practice.

The first experiment was conducted on a pool in the vicinity of Corvallis estimated to contain about 113,000 liters of water. This pool was a portion of a stream which dries up during the summer, leaving isolated bodies of water, probably connected by seepage through the sandy substratum. The pool contained patches of Spirogyra here and there together with a number of higher aquatic plants (Veronica, Cicuta, and others). The fauna included frogs, newts, and stickle-backs among vertebrates, and a great variety of insect life, the most abundant forms being Notonectids, Corisids, damsel fly larvae, neuropterous larvae, and beetles of various kinds, both adults and larvae. Five species of mollusks were present. *Physa occidentalis* and the small bivalve *Musculium walkeri* were abundant in the aquatic vegetation. *Fluminicola fusca* was abundant, and *Goniobasis plicifera* was fairly common on the sandy bottom, especially around the edges of the pool, and an unidentified Planorbis occurred sparingly in the vegetation.

On August 26, 113 gm. of commercial copper sulphate were dissolved in about 10 liters of water and sprinkled on the surface of the pool by means of a watering pot, making approximately a 1 to 1,000,000 solution, but without making any allowance for impurity of the copper sulphate, absorption of algae, combination with organic matter in solution, or dilution by seeping in of fresh water.

The effect of the experiment was studied 48 hours later. The masses of algae had been killed, but the higher plants, vertebrates, including the stickle-backs, and the various kinds of insects were apparently unharmed. No living specimens of Fluminicola or Planorbis could be found, though hundreds of dead ones were seen lying on the bottom. The majority of the Physae were dead, but a few seemed to be merely prostrate. Some specimens of Goniobasis were withdrawn into their shells and were evidently not dead. All the Musculium were lying on

the bottom with their shells tightly closed. Another examination was made on August 30, and at this time all specimens of Physa, Fluminicola, Planorbis, and Musculium and the majority of the Goniobasis were dead, but about one-third of the last had revived and were apparently well again. This fact was evidence that practically all the copper sulphate had been removed either by absorption by the algae or by the dissolved organic matter, increased by the disintegration of thousands of snails or by seepage through the sandy substratum, since it had previously been fully demonstrated that Goniobasis remained prostrate even in a 1 to 5,000,000 solution of copper sulphate.

A similar experiment was carried out on another pool of similar kind and with practically the same fauna and flora; this pool was, in fact, another isolated portion of the same stream. This time a copper-sulphate solution of 1 to 500,000 was made. All molluscs were apparently dead in 48 hours, and none subsequently revived. No other higher animals were affected at all.

To test the use of copper sulphate for destroying snails in a flowing stream, an experiment was attempted in Oak Creek, near Corvallis, Oreg. The water in this creek is cold and clear and flows rapidly. The stream is very uneven as to width, depth, and speed, consisting, in fact, of a series of sluggish pools connected by rapids and cascades. At this season of the year, September 1, the stream was very low, and was found to flow only about 550 liters per minute. The stream contained enormous numbers of *Goniobasis plicifera*, the bottom in some places being fairly covered with them.

To treat this stream a 7-gallon keg fitted with a drawn-out glass spigot which would feed a solution into the stream at an average rate of 1.5 liters per hour was filled with a copper-sulphate solution strong enough to make a 1 to 500,000 solution in the stream. This strength of solution was used to make allowance for combination with organic matter, precipitation in other ways, and error in estimation of the volume of the stream. The experiment ran smoothly for about 14 hours, and at the end of this time the snails for at least a mile down the stream were prostrate and apparently dead. Meanwhile, however, a rain storm came up which in the following 10 hours approximately tripled the volume of water in the stream. An attempt was made to strengthen the solution fed into the water at a corresponding rate, and this seemed to be successful. Pressure of other duties made it impossible to visit the experiment again until 48 hours later. At this time it was found that the spigot had been plugged by a particle of débris, though precautions had been taken to keep the solution as clear as possible. The cessation of flow had evidently occurred shortly after the experiment had last been visited, consequently the stream had been treated little more than 24 hours. A few of the snails were dead, but the majority had revived and were as active as ever.

On account of the writer's moving from Corvallis, Oreg., to Houston, Tex., a few days later, this experiment could not be repeated on Oak Creek, but a similar experimemt was made on a small stream or "bayou" a short distance from Houston. This stream, flowing about 1,500 liters per minute, is sluggish, fairly even in width and depth, and contains water moderately alkaline and rich in lime. The only abundant snail in the stream was a small Ancylus which occurs on dead leaves on the bottom. A few specimens of *Physa anatina* were obtained at each dredging.

To treat this stream a 10-gallon barrel was used, fitted with a glass spigot as before but protected from plugging up by the use of a glass funnel with the large end inside the barrel, this being covered with cheesecloth to strain the solution as it flowed out. The addition of a few cubic centimeters of sulphuric acid prevented the flocculent precipitation of iron sulphate, which is present as an impurity in commercial copper sulphate. The diminution in rate of flow from the spigot resulting from a lowering of the level of the fluid in the barrel follows a parabolic curve, in this case decreasing fairly steadily from 50 cc. to 30 cc. per minute until the barrel was half empty. To prevent a greater fall in pressure a 20-liter jar was placed above the barrel and connected with it by an automatic siphon, so that the contents of the jug would be utilized when the barrel was half empty. A simpler method would have been the utilization of a tube equal to the height of the barrel to give a greater head. By this method the entire contents of the barrel could be utilized before refilling without too great a change in the rate of flow of the copper solution. The experiment was allowed to run for 72 hours, although 48 hours' exposure to the copper solution had been found experimentally to be sufficient to kill snails. However, in a flowing stream it was thought advisable to give an extra day to make up for uneven flow and dilution in the deeper portions of the stream during the early part of the experiment and to give time for diffusion into the "dead" water along the sides of the stream. At the end of the experiment—that is, for the last 12 hours—the lower half of the barrel was allowed to run itself out, thus gradually diminishing the strength of the solution in the stream. It was thought that in this way the actual time during which the stream was treated by a full 1 to 500,000 solution would be at least 48 hours. Three days after the complet on of the experiment the stream was again dredged at intervals of about one-third of a mile at the same points at which dredgings were made prior to the experiment. A few empty Physa shells were found, but no living snails of any kind were obtained at any point along the length of the stream (about 1½ miles). It was unfortunate that the stream was not longer so that the actual distance over which the treatment was effective could be determined, but since this would

obviously vary greatly in different streams, according to evenness of width and depth, strength of current, purity of water, and possibly other factors, it would be necessary in treating any stream for the destruction of snails to determine, after the experiment, the distance over which it is effective and to repeat the experiment at a point on the stream a little above where the first live snails were found.

By utilizing a 50-gallon barrel and filling it at 12-hour intervals with a 10 per cent solution, streams running as much as 3,500 gallons per minute could be treated by this method, and, of course, by the use of several such barrels, still larger streams could be treated. Repeated attempts were made to find a method by which the copper salt could be fed into a stream at a constant rate without first being put into solution. This would, of course, save much time and labor in the treatment of large streams. A method was finally worked out by which it was hoped that this could be accomplished. Cylinders of sheet metal were carefully lined with paraffine inside to prevent any chemical action with the copper sulphate. Wooden tubes could be used as well but are not so readily obtainable as are the sheet metal tubes, which, in diameters of from 2 inches up, can be obtained from any tinsmith. A copper or bronze screen is tied over the end of the tube, and the tube is filled with copper-sulphate crystals of more or less uniform size. Commercial "pea" crystals could be used, or crystals of desired size can be obtained by sifting through two screens. The screened end of the tube is immersed about 1 cm. in the stream to be treated, and the copper sulphate is dissolved out from the bottom of the tube, a fresh supply being constantly furnished by gravity in the tube. Theoretically the copper salt should go into solution at a fairly constant rate, determined by the area exposed to the water, the speed of the stream, and the temperature of the water. Up to the present, however, it has not been found possible to make this simple apparatus work satisfactorily in practice, because of the fact that all the water in the vicinity of Houston is strongly alkaline. The alkalinity precipitates the iron sulphate contained as an impurity in commercial copper sulphate and also forms, in the course of two or three hours, considerable deposits of copper carbonates. These two substances together tend to clog the screen through which the copper sulphate is taken into solution, thus causing a rapid diminution in the rate of solution. If this difficulty could be overcome by some feasible method of keeping the water at the mouth of the tube slightly acidified, or if the water to be treated were not alkaline, large streams could be treated with comparatively little trouble by this method, using several tubes of suitable diameter at intervals across the streams. It would, of course, be preferable to treat streams at a comparatively shallow, rapid-flowing point, since this would facilitate a rapid diffusion throughout the water.

SUMMARY AND CONCLUSIONS

(1) Fluke diseases of both man and domestic animals are of great importance in many parts of the world. They are debilitating diseases of long duration and difficult to treat or cure. Preventive measures, therefore, are of great importance. The working out of preventive measures based on scientific facts has only recently become possible, since the life histories and modes of infection of the human flukes have been discovered only in the last three or four years.

(2) In all known cases fresh water snails act as intermediate hosts for the important flukes of man and domestic animals. A practical and efficient method of destroying these snails would make the ultimate eradication of fluke diseases, in spite of the difficulty in treating them, a matter of brighter prospect than the eradication of hookworm and other intestinal parasites, in which the sanitary disposal of feces must be relied upon.

(3) Experiments by the writer, carried out to find some cheap, harmless method of treating water to destroy snails, demonstrated that copper salts exert a powerful toxic effect upon snails even in very high dilution. In an experiment upon eight species of six families it was demonstrated that copper sulphate in proportions of 1 part to from 500,000 to 2,000,000 parts of water destroys snails of all these species within 48 hours; 50 per cent or more are destroyed in dilutions up to 1 to 5,000,000. From the point of view of expense, harmlessness, and convenience in use copper sulphate is preferable to any other substance which has been tried or suggested for destroying snails. The eggs of the snails are not destroyed by the copper salts.

(4) Copper salts are also highly toxic to algae, fungi, and other lower organisms but are apparently harmless, in the dilutions used, to higher plants and animals, except fish. Water treated with copper sulphate, therefore, is uninjured for drinking, bathing, or irrigation purposes.

(5) The effectiveness of copper sulphate in water is modified more or less by temperature, alkalinity, dissolved organic matter, and living algae. Some allowance should be made for these factors in estimating the amount of copper to be used in any given body of water. The proportion should vary from 1 to 1,000,000 in relatively pure water at 20° C. or above to 1 to 500,000 in water which is very cold, is alkaline, contains dissolved organic matter, or harbors an abundance of algae. If the growth of algae is very luxuriant, it would probably be advisable to kill these algae by a preliminary treatment with a 1 to 1,000,000 solution of copper sulphate, following this in the course of a few days or a week by a second treatment.

(6) Copper sulphate can be administered to ponds, reservoirs, or other bodies of standing water in the way advised by Moore and Kellerman for the destruction of algae in water. This method provides for the

solution of the correct amount of the salt from a sack attached to the back of a canoe or boat, or, in very small pools, to the end of a pole. Dissolved copper sulphate can conveniently be sprayed on small pools from a spray pump or even an ordinary garden watering pot. In most cases Bullinus, Physopsis, Planorbis, and Limnaea could be destroyed by these methods.

(7) For the treatment of running streams the use of a barrel of suitable size, fitted with a screened spigot, is recommended. The barrel is filled with water, and sufficient copper sulphate is dissolved into it so that the desired amount will be fed into the water per hour. Inasmuch as no two spigots will flow at exactly the same rate and since the rate of flow will diminish as the level of the fluid in the barrel is lowered, it is necessary to determine beforehand the rate of flow at different levels and to calculate the amount of copper sulphate to be dissolved according to the average rate of flow. By the use of a tube of equal or greater length than the height of the barrel, so that the head is increased, the diminution in rate of flow can be greatly lessened. The addition of a few cubic centimeters of sulphuric acid to the solution in the barrel prevents the precipitation of iron sulphate, which is present as an impurity in commercial copper sulphate and tends to clog the filter. Melania and Blanfordia would probably have to be attacked by this method, since they live in flowing water.

(8) In water which is not alkaline, large streams could be treated more easily by allowing the copper sulphate, in the form of uniform crystals, to dissolve directly into the stream through the screened end of a tube. The amount of salt which would go into solution per unit of time would depend on the diameter of the tube, the speed of the stream, and the temperature of the water. If some feasible method could be devised for slightly acidifying the water at the point where solution of the salt is taking place, this method could be used advantageously in all but very small streams.

(9) It is believed that by attacking the intermediate hosts of the various pathogenic flukes of man and domestic animals by the use of copper sulphate as herein outlined trematode diseases can successfully be brought under control and can either be greatly reduced or entirely eliminated in endemic areas, and this with comparatively little expense and without active cooperation on the part of natives. With Government aid and supervision, the work being carried out under the direction of scientifically trained men or commissions, it seems entirely possible that entire States or countries, at least in the vicinity of towns and villages, could be freed of human fluke diseases, and that seriously affected districts where sheep and cattle are raised could have the fluke scourge wiped out in a short time with little expense.

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INJURY TO SEED WHEAT RESULTING FROM DRYING AFTER DISINFECTION WITH FORMALDEHYDE

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INTRODUCTION

Much has been written on the use of formaldehyde as a fungicide for wheat and other grains infested with smut, but relatively little has been carefully done on the effect of such treatment on the seed. The usual recommendation has been a dip of about 10 minutes in a solution consisting of 1 part of commercial formaldehyde solution to 320 parts of water, followed by a 10-minute drain. Almost without exception instructions are given to dry the seed thoroughly before storing it. The frequent advice that it be sown immediately after treatment and not stored indicates that it has been learned by experience that injury to the grain occurs not so much from the treatment as from holding it in storage afterward. However, it has been almost universally concluded, without experimental evidence, that damp storage causes the injury. Thus, practically every publication dealing with seed treatment carefully warns against the storage of formaldehyde-treated seed that has not been thoroughly dried after treatment.

The present investigation of the post-treatment action of formaldehyde on seeds was begun in 1918 in the plant pathology laboratories of the University of California as a part of the cereal-smut eradication campaign carried on by the United States Department of Agriculture and was continued through a period of nine months. The major conclusion reached is that it is extremely hazardous to dry seed which has been treated with formaldehyde solution,[2] and that, contrary to common belief, seed wheat is absolutely uninjured by a 0.1 per cent solution (1 to 40) and, if kept moist, may be held indefinitely without injury, unless attacked by molds. We believe that the data here presented will contribute to our knowledge of the physical and chemical properties of formaldehyde and the relation of these properties to physiological processes in the seed. Such knowledge will undoubtedly

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[2] Reports sent in to Dr. H. B. Humphrey and to Prof. W. W. Mackie of occasional poor stands of wheat from treated seed sown by farmers in the dry regions of California and Oregon indicate that field results confirm those arrived at through these experiments.

be helpful in any consideration of the more practical problems connected with the use of this chemical as a fungicide.

Certain investigators working on this problem have shown that injury to formaldehyde-treated seed occurs when the seed is allowed to dry after treatment. The earliest report we have found of such work is that of McAlpine (*11*),[1] whose experiments showed that seed treated with a solution of 1 pound of formaldehyde in 40 gallons of water just prior to sowing under conditions favoring immediate germination grew as well as untreated seed. If, however, the seed was allowed to dry for a day or more before germinating or if it remained in dry soil some days before a rain, it suffered extreme injury. He gives instances of such injury reported by farmers who from experience had learned to sow formaldehyde-treated seed in moist soil immediately after treating. McAlpine attributed this injury to the hardening effect of formaldehyde on the seed coat. He claimed that by soaking the dried treated seed in water prior to sowing this injury was averted. He further stated that the injury after a dip in a 1 to 40 solution was most pronounced when the seed had been kept a week after treatment. After two weeks it began to improve until, when sown a month after treatment, it was practically as good as 24 hours after treatment. He stated also that this recovery did not occur when the solution used was twice as concentrated.

In 1908, Shutt (*14*) found that a delay of three days in sowing after the formaldehyde treatment reduced the percentage of germination and increased the proportion of weak and slender plants. In opposition to this are the results reported by Hurst (*8*), who states that seed may be—

> treated and kept for any reasonable length of time without affecting its vitality.

Some of his samples, he says, had been treated 12 months before and germinated as well as the untreated seed. Stewart and Stephens (*16*) found that after the use of a 1 to 50 solution their samples were uninjured by 6 weeks' dry storage, which was the longest storage period tested. Brittlebank (*3*) noted a falling off in the germination of seed treated with formaldehyde solution after being kept dry a week, the decrease continuing to the sixth week, after which the percentages rose and fell with various fluctuations through the remainder of the 54 weeks. Güssow (*6, p. 21–22*) reported some figures obtained by Dr. C. E. Saunders, Dominion Cerealist, showing that treated seed which originally germinated 75 per cent was entirely killed after being stored dry a year. Some barley and oats treated similarly were almost wholly killed after standing dry a year.

The first investigators to connect this storage injury with that property of formaldehyde by virtue of which it forms a solid condensation product or polymer upon evaporation were Darnell-Smith and Carne (*5*), who

[1] Reference is made by number (italic) to "Literature cited," p. 243–244.

attributed the conflicting reports of the injury resulting from the formaldehyde treatment to variations in the deposit of this polymer on the seed as it dried. They found low germination percentages and defective seedlings to result from the drying of treated seed. Their results do not agree with those of McAlpine, which were responsible for the latter's conclusion that soaking in water prior to sowing removed the cause of injury. They did find, however, that washing immediately after treatment prevented subsequent injury in storage by removing the source of the deposit. They thought that there was no internal poisoning of the seed before germinating but that there was some deleterious chemical action of a formaldehyde salt in the pericarp, which was alleviated by soaking. Müller and Moltz (*12*) proved that the polymer, paraformaldehyde, when mixed with the soil was very injurious to wheat sown in it.

An interesting and comprehensive report on the secondary effects of formaldehyde treatment is the recent article by Kiessling (*9*). He obtained severe injury upon storing treated seed which had been dried, and this injury he found to be cumulative as the duration of the storage period continued. He also was unable to confirm McAlpine's statement that soaking the dried seed before sowing prevented the injury. Although giving adequate and convincing proof that dry storage is more fatal than damp storage, he does not advance any explanation.

Coons (*4*) also found that it is unwise to hold formaldehyde-treated grains any length of time and that the injurious action is cumulative when the treating solution is dried on the seed. He suggests that this injury may be due to the formation of the solid condensation product, paraformaldehyde, which might persist on the grain even after months of drying.

POST-TREATMENT ACTION OF FORMALDEHYDE ON DRYING SEED WHEAT

Except the studies of Coons (*4*) and those of Stewart and Stephens (*16*), it will be noted that all the work on dry-storage injury to wheat has been done outside the United States. This no doubt accounts for the fact that it has been generally overlooked in this country or at least has not resulted in any modification of the widespread instructions relative to drying formaldehyde-treated wheat before storage. It was to investigate this supposed formaldehyde injury to damp stored seed that the studies here recorded were begun. These experiments resulted in the rediscovery of the fact that so long as the seed treated with a 0.1 per cent (1 to 40) solution remains damp there is no injury from the chemical but, when dried, the seed is variously injured, depending upon the manner of drying and upon the moisture content of the atmosphere surrounding the seeds.

In the following experiments the seeds were left for 10 minutes in a 0.1 per cent solution of formaldehyde followed by a draining period of 10 minutes. This strength is equivalent to 1 pint in 40 gallons of solution, varying in small degree from that commonly referred to as 1 to 40, which means 1 pint of standard formaldehyde solution in 40 gallons of water. As the formaldehyde solution used in the laboratory contained 36.2 per cent formaldehyde, such a dilution would be 1 part of formaldehyde in 884 parts of solution, or 0.113 per cent. Unless otherwise stated, the wheat used was Little Club with a low percentage of thrashing injury. After treatment the seed was spread on towels for an hour in order to remove excess surface moisture. The damp seed was then divided into two lots. One lot was put into three Mason fruit jars, holding about a quart each, and sealed. The other lot was put into three boxes, 4 by 5 by 6 inches, and left uncovered. They were stirred frequently throughout the experiment. These boxes each contained the same quantity of wheat as did the jars. The original idea in having three samples of each seed lot was to determine the relation of temperature to the injury which was expected to appear in the damp samples. One box and one sealed jar were left in the refrigerator at a temperature of 10° C., one of each in the laboratory at 20°, and one in the greenhouse, where the temperature averaged about 30°. For each of the six samples, as in all subsequent experiments, there was a control of seed dipped in water instead of formaldehyde.

The following germination tests were made on blotters placed in square pans, 12 by 12 inches, 1¼ inches deep, kept at room temperature. The pans were covered with square pieces of glass, which made it easy to observe the progress of the germinations. The depth of the pans gave the seedlings a chance to grow erect and more normally than would be the case if they were grown between blotters. Only those seeds were called germinated which produced both a root and plumule. Many which did so were too severely injured to produce plants in soil, but the approximate percentage of these was obtained by contemporaneous soil germinations (Table II). Soil germinations have the advantage of approximating more closely field results. The many advantages in the use of blotters, however, lead the writer to emphasize the fact that they are just as valuable to show the occurrence and comparative degrees of seed injury. In view of the possibility of earlier detection and easier study of such injury, they even may be preferable. The results of the blotter germination tests are given in Table I.

TABLE I.—*Percentage of germination of wheat treated with 0.1 per cent formaldehyde solution and variously stored*

Treatment and storage.	Stored 0 days.	Stored 1 day.	Stored 3 days.	Stored 6 days.	Stored 10 days.	Stored 14 days.	Stored 21 days.	Stored 28 days.	Stored 35 days.	Stored 42 days.	Stored 60 days.	Stored 120 days.
Stored in refrigerator at 10° C.:												
Treated, stored dry	100	100	98	[a] 90	[a] 100	[a] 92	[a] 92	[a] 74	[a] 74	[a] 72	[a] 52	[a] 10
Control, stored dry	99	100	100	98	100	98	98	98	98	100	100	98
Treated, stored damp	100	98	92	90	98	92	100	94	98	100	[b]	[b]
Control, stored damp	99	98	98	100	100	98	100	100	96	[b]	[b]	[b]
Stored in laboratory at 20° C.:												
Treated, stored dry	100	100	[a] 92	[a] 92	[a] 84	[a] 72	[a] 76	[a] 86	[a] 72	[a] 64	[a] 60	[a] 32
Control, stored dry	98	98	98	100	98	100	100	98	100	98	100	98
Treated, stored damp	100	98	98	98	100	98	98	[b]	[b]	[b]	[b]	[b]
Control, stored damp	99	100	98	100	98	[b]	[b]	[b]	[b]	[b]	[b]	[b]
Stored in greenhouse at 12° to 35° C.:												
Treated, stored dry	100	98	[a] 90	[a] 90	[a] 84	[a] 96	[a] 90	[a] 90	[a] 96	[a] 98	[a] 90	[a] 82
Control, stored dry	99	100	94	100	98	94	100	100	96	98	100	98
Treated, stored damp	100	96	94	98	100	94	100	100	100	98	100	94
Control, stored damp	99	100	100	100	100	92	96	[b]	[b]	[b]	[b]	[b]

[a] These seedlings were markedly retarded and injured, as indicated by slow germination and deformed plumules. The lower the percentage of germination the more extreme is this malformation of those which succeed in producing the plumule and root. Therefore, the percentage figures for the dried, treated seeds, especially those from the greenhouse, do not indicate the real extent of their injury.

[b] Attacked by molds.

The outstanding fact shown by these experiments is that all the seed which was treated with the formaldehyde solution and then dried by being allowed to stand open to the air was either killed or seriously injured after three to six days, while that treated at the same time and stored at the same temperatures, but kept damp by being sealed in jars, was practically uninjured up to the time it was destroyed by molds. Later experiments have shown that injury may appear in dry-stored seeds in less than three days, depending on the manner of drying. The dry controls maintained the original germination throughout, and the wet ones did also until they were killed by the development of fungi in the jars. It will be noticed that molds appeared more slowly in the damp, treated seed than in the damp controls, giving evidence of the fungicidal action of the formaldehyde remaining on the seed. The reason for the more extreme injury in the lots stored at room temperature and in the refrigerator compared with those in the greenhouse will be discussed later. These percentages also show most strikingly that the injury to dried seeds is cumulative and that there is no recovery. This is borne out by all subsequent experiments and refutes the claim of McAlpine (*11*) and Darnell-Smith and Carne (*5*) that there is a steady improvement after the extreme injury which appears after a week or so.

In addition to low germination percentages, the injured samples showed a characteristic deformity and extreme retardation of the injured seedlings. The earliest appearance of injury in the dried seeds was simply a noticeable retardation of germination in the samples after being stored three and six days, the plumules and roots never catching up with those of the uninjured seedlings. The retardation became more extreme as storage continued, with an ever-increasing number of short plants which grew very slowly and resulted in stunted and misshapen plumules and underdeveloped roots. After 10 days' storage all the seeds of the three treated and dried lots were thus inhibited, so that upon germinating they presented the appearance shown by those in Plate 36, A. The characteristic deformity by which this extreme formaldehyde injury can always be detected is the curving of the plumule as it emerges until it is sickle-shaped (Pl. 36, B). The growth of the sheath is inhibited so that it never grows more than a few millimeters, leaving the young leaves to push out unprotected, spindling, and weak, unable to push their way through soil. The roots are underdeveloped but show no deformity. It has been noted throughout these experiments that the greater the retardation of germination in any injured seed lot, the greater the proportion of weak, spindling plants produced. Whether the effect of the formaldehyde on the sheath is to stop growth by stopping cell division or by inhibiting the growth of the cells after they have divided was not determined.

Anyone observing the seeds of the injured dry lots, the uninjured damp ones, and the controls, germinating in blotters where invasion by Rhizopus

was possible, would notice at once the luxuriant growth of mycelium on the injured seeds and its comparative rarity on the uninjured ones. He might be inclined to ask whether the injury of the former samples was not the result of fungous activities instead of action of formaldehyde which might by its presence simply stimulate the growth of the mold. This question is easily answered by disinfecting some of the dried, treated seeds by a 10-minute dip into a 1 to 1,000 solution of mercuric chlorid and germinating them on sterile blotters. The seedlings show the same characteristic injury, but the percentages of germination are higher, though not normal. This is because when they escape infection some of the injured seeds succeed in germinating and produce weak plants. These seeds, had they not been disinfected, would have been killed by the invading fungus before the retarded root and plumule could emerge. The extent of the development of this fungus on the various lots of germinating seeds serves as a fairly accurate index of the injury done to the seed by the treatment. It is concluded from such experiments and many others showing the same fact, which will be reported in detail in a subsequent paper, that injury from drying after the formaldehyde treatment predisposes the seed to attack by molds, especially Rhizopus, the chemically injured embryo being unable to resist infection.

It is commonly believed that blotter germinations are worthless so far as being an indication of the viability of seeds in soil. Therefore, along with the blotter germinations summarized in Table I, occasional tests of the stored seeds were made in pots of sandy loam soil in the greenhouse. It was found that with the uninjured samples the soil germinations gave the same results as those made at the same time in blotters. With injured seeds they were lower, as was to be expected, for in the blotters all those seeds were counted germinated which produced both root and plumule even though these were stunted or deformed. In the soil such seedlings would never reach the surface, and so the count of germinated plants from injured seed lots would be lower. Consequently, the injury produced by drying the formaldehyde treated seeds appeared even more strikingly in the soil and would more closely approximate actual field results. This is shown in Table II.

These figures do not indicate the full extent of the injury suffered by the dried treated seed. Many of the seedlings from the injured samples are short and spindling, while none of this sort are found in the controls or in the samples which had been stored damp (Pl. 37, A). This same extreme injury was shown by the seeds stored dry in the laboratory, but the figures are not included in Table II, because the damp controls of both the untreated and the treated seed were destroyed very quickly by the rapid development of Penicillium and Aspergillus at that temperature. Plate 37, B, shows the seedlings produced by these three seed lots injured by drying and the seedlings produced by two of the controls.

TABLE II.—*Percentage of germination in potted soil of wheat treated with 0.1 per cent formaldehyde and stored for various periods*

Treatment and storage.	Stored 10 days.	Stored 32 days.	Stored 56 days.
Stored in refrigerator at 10° C.:			
Treated, stored dry	62	28	18
Control, stored dry	100	100	100
Treated, stored damp	100	[a] 92	[a] 90
Control, stored damp	100	[a] 92	[a] 76
Stored in greenhouse at 15° to 35° C.:			
Treated, stored dry	60	54	74
Control, stored dry	98	100	98
Treated, stored damp	96	96	100
Control, stored damp	96	[a] 94	[a] 82

[a] The germination of these samples is lowered by the development of molds in the jars. As will be reported in a subsequent paper, saprophytic fungi attack stored wheat whenever the humidity is 70 per cent or more, the treated seeds being attacked more slowly because of the slight protection afforded by the formaldehyde.

After it had been determined that wheat stored and allowed to dry after treatment was seriously injured, the next question which arose was whether the same injury would be produced if seed sown immediately after treatment in dry soil remained there for some time before sufficient rain fell to dampen the soil and induce germination. In dry regions wheat often lies in the soil for weeks before germinating. To duplicate these conditions, seed was treated in the usual manner with a 0.1 per cent solution of formaldehyde and sown, 50 seeds in a pot, in air-dry soil. On one series, a 0.2 per cent solution was used to show more strikingly the cumulative nature of the injury. One pot of each, with a control of seed treated similarly with water, was watered after predetermined intervals such that the first pot was watered and started to germinate immediately after planting while the last one remained dry for a month. The results of the experiments with wheat are given in Tables III and IV.

TABLE III.—*Percentage of germination of Little Club wheat after lying in dry soil (Yolo clay loam) following treatment with 0.1 per cent formaldehyde solution*

Treatment.	Water applied after—								
	0 days.	1 day.	2 days.	3 days.	4 days.	5 days.	7 days.	10 days.	14 days.
Treated	98	94	94	86	94	64	52	52	42
Controls, soaked in water	100	98	100	98	98	98	98	98	100

The data in Table III indicate that it is not safe to treat wheat with formaldehyde, even when the strength of solution is as weak as 0.1 per cent, if the seed must be sown in very dry soil without certainty of rain within a few days.[1] Besides a lower percentage of germination, the ger-

[1] Field reports are found to be in agreement with these laboratory tests. The hitherto unexplainable poor stands of wheat from treated seed obtained by the farmers of the dry regions of California can now be safely attributed to the fact that the seed lay in the dry soil for some time before rain.

mination of the injured seed lots was retarded, often several days, and they produced a considerable number of spindling or short plants which apparently never would be strong (Pl. 38, A).

The injury from drying, either in storage or in the soil, is greater the more concentrated the solution used. The data given in Table IV demonstrate this fact, the experiment differing from that summarized in Table III only in the use of sandy-loam soil instead of the heavy Yolo clay loam and in the fact that a parallel experiment was run at the same time in which some of the treated seed was kept in a box in the laboratory and a sample was germinated in blotters after drying for periods corresponding to those in the soil experiment (Pl. 38, A).

TABLE IV.—*Percentage of germination of Little Club wheat treated with formaldehyde and dried, both in the soil and in the air*

Length of drying period.	Sown in dry soil.			Dried in the air and germinated in blotters.		
	0.1 per cent formaldehyde solution.	0.2 per cent formaldehyde solution.	Control, dipped in water.	0.1 per cent formaldehyde solution.	0.2 per cent formaldehyde solution.	Control, dipped in water.
Days.						
0	100	92	98	98	98	96
2	84	68	.....	98	98	100
5	84	66	.....	90	60	100
7	80	60	.....	84	66	100
10	86	48	.....	86	50	96
14	74	34	.....	90	52	96
20	88	44	.....	62	54	96
30	62	48	100	80	44	96

In none of the experiments summarized in Tables I to IV was there any injury to seed germinated at once after the dip into either 0.1 per cent or 0.2 per cent formaldehyde. This fact is not in agreement with results reported by many experimenters. Stewart and Stephens (*16*), for instance, found that an immersion of 10 minutes in a 1 to 40 solution (0.1 per cent) caused almost a 50 per cent loss. Kiessling (*9*), for example, notes the great variation in the results reported on the effect of formaldehyde on germination of seed. He concludes from the work of others and from his own experiments that formaldehyde produces a serious effect on the seed, the degree of injury depending on the sensitiveness of the different varieties and the condition of the sample. None of the wheat varieties tested in this laboratory (Little Club, Early Baart, Marquis, Defiance, Sonora, and White Australian) was ever found to be injured in the least by the recommended treatment, or by one twice as strong, whether germinated in blotters or in the soil, so long as it was sown immediately after treatment. Not only will the seed be uninjured by the usual 20-minute exposure to a 0.1 per cent solution but it will

stand an immersion of 8 hours without injury. It can remain 1 hour without injury in a solution twice as strong. Table V shows the result of an experiment to determine the resistance of Little Club wheat to long exposures to various strengths of formaldehyde solutions.

TABLE V.—*Relation between strength of solution, duration of exposure, and seed injury*

Strength of solution.	Soaked 20 minutes.		Soaked 1 hour.		Soaked 6 hours.		Soaked 8 hours.		Soaked 24 hours.	
	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.
	Per ct.	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*
Saturated	0	.....	0	.....	0	.....	0	.....	0	.....
4.50 per cent	100	3.5	25	0.2	0	.....	0	.....	0	.....
0.45 per cent	95	3.5	90	.6	0	.....	0	.....	0	.....
0.20 per cent	100	3.5	95	3.5	40	1.0—	15	1.0—	0	.....
0.10 per cent	100	3.5	95	3.5	100	3.5	95	3.5	85	2.5
Control, untreated	100	3.5	95	3.5	100	3.5	95	3.5	95	3.5

Table V shows that Little Club wheat, thrashed with little injury, will stand an 8-hour exposure to a 0.1 per cent solution, a 1-hour exposure to a 0.2 per cent solution, or a 20-minute exposure to 0.45 per cent and 4.5 per cent solutions.

The post-treatment injury from dry storage after subjection to a 0.1 per cent solution as well as to stronger ones has been demonstrated not only with Little Club and Early Baart wheat but with Sonora, Marquis, Defiance, and White Australian.

PHYSICAL PROPERTIES OF FORMALDEHYDE AND PARAFORMALDEHYDE

After the fact had been established that a 0.1 per cent solution is innocuous but that the drying of this solution on the seed is harmful, the next step was to investigate the physical and chemical properties of formaldehyde in order to find a cause for the injury and a means of avoiding it. The natural supposition was that the injury is due either to a concentration of the solution on the seeds as they dry or to a coating of paraformaldehyde left upon them as the solution evaporates. It seemed at first inexplicable, however, that the seeds stored damp, or even wet, should remain absolutely uninjured indefinitely. In an effort to connect these facts with the possible persistence and disappearance of the chemical on the seed some qualitative tests for formaldehyde in washings of the damp and dried seed were undertaken. It was the result of these first qualitative tests which led to the intensive study of the behavior of formaldehyde solution and paraformaldehyde and the possible determination of the cause of seed injury reported in this paper.

To detect the presence of formaldeyde on treated seed, Tollen's "silver mirror" aldehyde test [1] was used. To obtain comparable water extracts of the seed lots a uniform procedure was adopted which consisted in extracting 15 cc. of the wheat sample with 10 cc. of distilled water for two minutes in a 100-cc. graduated cylinder which was rotated and shaken constantly to wash all the seeds as thoroughly as possible. Five cc. of the washings were then transferred to a test tube by means of a pipette. Extracts of all the wheat samples to be studied were thus prepared before proceeding. This is because it was necessary to add the reagent to all at as nearly the same instant as possible in order that results given by color changes might be comparable, since it is by the relative rapidity of their appearance that the relative quantities of precipitate formed by the presence of formaldehyde are shown. One cc. of Tollen's reagent was then added quickly to each tube by means of a pipette, and the tubes were watched for the appearance of the black, or, at first, dark brown precipitate indicating the presence of formaldehyde. The relative quantities of formaldehyde present in the tubes were shown by the rapidity of formation and by the density of this precipitate.

Several interesting facts were disclosed by the application of this test to the washings of treated seed. In the first place, distinct and positive reactions were invariably obtained from seed which had been drying for weeks, thus giving a clue to the reasons for the cumulative injury suffered by seeds in drying. Positive reactions were given by extracts of samples, the germinations of which were reported in Tables I and II, after the seed had dried nine weeks in the laboratory. This, however, was longer than the average persistence of the paraformaldehyde, which, on account of its volatility, usually disappeared in a month, depending on the conditions of drying. It is understood, of course, that, in the presence of moisture, paraformaldehyde at once breaks down and is again formaldehyde in solution.

In addition to this proof of the persistence of formaldehyde on the seed in the form of paraformaldehyde, the qualitative tests showed invariably that about 24 hours after treatment there was more formaldehyde on the seed stored damp in a sealed jar than on that treated at the same time and stored dry, showing a diminution in the quantity as the seed dried. After 48 to 72 hours, the amount on the seeds in the sealed jars had diminished at a more rapid rate, so that extracts from them gave weaker and slower reactions than those from the dried seed. Within a

[1] Tollen's reagent is an ammoniacal solution of silver nitrate which when added to a dilute aldehyde solution produces a black precipitate or, upon standing and in the presence of a sufficient amount of the aldehyde, forms a silver mirror by the precipitation of metallic silver on the sides of the test tube or other container. It is made by dissolving 3 gm. of silver nitrate in 30 gm. of water and 3 gm. of sodium hydroxid in 30 gm. of water, the two solutions being kept separate until ready for use, when they are mixed in equal parts by volume and the resulting precipitate of silver oxid is dissolved by the addition, drop by drop, of ammonia (specific gravity 0.923).

week, or at most two weeks, the damp seed ceased entirely to give any formaldehyde reaction. An odd reddish brown color resulted when Tollen's reagent was added to these extracts, but there was no black precipitate. The question was to determine where the formaldehyde had gone, for it seemed extremely inconsistent that it should disappear in a sealed jar and yet remain on seed open to the air. The answer was suggested by Dr. C. W. Porter, organic chemist at the University of California, who said that it probably was absorbed by bacteria and mold growing in the damp wheat.

To determine whether this were the case, some treated seeds were divided into several lots. Part were inoculated with the spores of Penicillium and sealed in small jars. The rest were left uninoculated and stored similarly. Within a few days extracts of the former samples ceased giving the formaldehyde reaction and produced the peculiar reddish brown color noted above. The uninoculated lots continued to show the presence of the chemical for some days longer but eventually became moldy and then gave the same reddish brown color with the ammoniacal silver nitrate.

Having demonstrated the persistence of formaldehyde on drying seed and its disappearance from seed stored damp, and having evidence pointing to the fact that seed injury from this fungicide may be dependent on the formation of paraformaldehyde on the seed, we next undertook a more critical study of the evaporation and polymerization of formaldehyde solutions.

It was found, upon evaporating the undiluted commercial solutions, that a surprisingly large quantity of the solid, white, condensation product was produced from comparatively small volumes. The percentage by weight of the solid formed varied greatly in different determinations because of variations in the conditions affecting the rate of evaporation—namely, quantity of solution, area of free surface, atmospheric humidity, temperature, etc. Even with these factors controlled, the same percentage could not be obtained with successive determinations because there is continuous evaporation of the solid paraformaldehyde after it has formed, as well as of the moisture in the, at first, waxy residue. In our determinations a procedure as nearly uniform as possible was always followed—that is, 50 cc. of undiluted 36.2 per cent formaldehyde solution were evaporated by exposure to the air in a 100-cc. evaporating dish, the residue being allowed to dry until the yellow color and waxy texture had disappeared. The dry residue was weighed as soon as possible after it became pure white, brittle, and easily powdered. A solution analyzed at the Insecticide Laboratory of the University of California and found to contain 36.2 per cent formaldehyde (specific gravity 1.090) produced under these conditions an average of 9.85 gm. of paraformaldehyde per 50 cc. This is 18.07 per cent of the weight of

the solution $\left(\frac{9.85}{50 \times 1.090} = 18.07\right)$ and 49.92 per cent of the weight of the formaldehyde present $\left(\frac{9.85}{0.362 \times (50 \times 1.09)} = 49.92\right)$. A 20-cc. volume of undiluted formaldehyde solution gave 16.1 per cent paraformaldehyde by weight of the solution and 44.6 per cent by weight of formaldehyde originally present in it. A 10-cc. volume, evaporated under the same conditions as the other two, gave only 7.8 per cent by weight of the solution and 21.5 per cent by weight of formaldehyde. From this and other data we know that the quantity of paraformaldehyde appearing as residue upon the evaporation of a formaldehyde solution depends on the original volume evaporated. Rate of evaporation is probably the determining factor, the extent of the evaporating surface being small in proportion to the volume as the latter is increased.

It has been shown (*10*, *14*) that dilute formaldehyde solutions grow stronger as evaporation proceeds. Notwithstanding this fact, published statements to the contrary occur in literature relating to the use of formaldehyde as a fungicide. The weakest solution analyzed by the writer was a 0.113 per cent dilution. It was found by quantitative analyses[1] of solutions before and after evaporation that the amount of formaldehyde per cubic centimeter of solution steadily increased as evaporation proceeded. Some was lost with the water, as, otherwise, the amount in the last 5 cc. would have been considerably larger than it was. The increased concentration was great enough to indicate a deposit of paraformaldehyde upon complete drying. As shown by the following test, this proved to be the case. A 0.1 per cent solution of formaldehyde was made with distilled water, and 50 cc. were put in each of two 8-cm. evaporating dishes and evaporated by leaving them exposed to the air of the laboratory, together with two controls containing 50 cc. each of distilled water. As soon as the dishes were absolutely dry (in 12 days) each dish was rinsed with 5 cc. of hot distilled water, and the washings were poured into test tubes. To each was added 1 cc. of Tollen's reagent. Results were distinct and decisive, a dark brown color appearing in the

[1] The most accurate and convenient method found for determining quantitatively the amount of formaldehyde in a solution is that of Romijn (*13*). To 5 cc. of the formaldehyde solution are added 5 cc. *N*/10 iodin solution and so much strong sodium hydroxid solution, drop by drop, that the liquid assumes a light yellow color. After a period of 10 minutes the solution is acidified with hydrochloric acid and the free iodin is titrated back with *N*/10 sodium thiosulphate solution. Every cubic centimeter of the iodin which has been used up in the reaction with formaldehyde (the difference between the original 5 cc. added and the amount left to react with the sodium thiosulphate) represents 0.001501 gm. of formaldehyde present in the solution.

The analyses, repeated several times with approximately the same results, were obtained by evaporating 100 cc. of a 0.1 per cent solution at room temperature in an 8 cm. evaporating dish. The quantity of solution used, atmospheric humidity, and other factors determine the degree of concentration of the evaporating solution at any point in the process. In the first analysis the amount of formaldehyde per cubic centimeter of solution increased from 0.0055 gm. to 0.0069 gm. after the solution had evaporated from an original volume of 100 cc. to 6 cc. (in 8 days). In a second analysis the increase was from 0.0058 gm. to 0.0069 gm. per cubic centimeter, the evaporating solution decreasing in volume from 100 cc. to 10 cc. in an equal length of time.

washings of the formaldehyde dishes, while the controls remained colorless. This showed that paraformaldehyde is left as a residue on the evaporation of solutions as weak as 0.1 per cent.

By successive weighings of the same sample it was found that paraformaldehyde is volatile, gradually breaking down and escaping as formaldehyde gas. To this property we may safely look for a large part of the seed injury following treatment with formaldehyde. Figure 1 illustrates graphically the rate of decreasing weight of 10.54 gm. of paraformaldehyde exposed to the air of the room in an 8-cm. evaporating dish in which it was originally formed by the evaporation of 50 cc. of a 36.2 per cent solution.

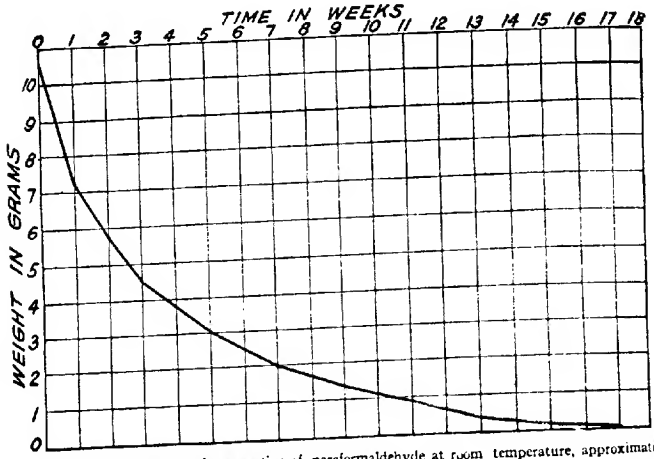

FIG. 1.—Graph showing rate of evaporation of paraformaldehyde at room temperature, approximately 20° C.

INJURIOUS EFFECT OF PARAFORMALDEHYDE ON SEEDS

After it had been demonstrated that a solid residue is left upon the evaporation of a formaldehyde solution and that this substance is constantly breaking down to form formaldehyde gas, it seemed probable that the cause of injury to treated seeds upon drying was the production of an atmosphere of concentrated gas adjacent to the seed as a result of the constant evaporation of this coating of paraformaldehyde. The gas, being heavier than air, would tend to remain around the seeds, especially when they are dried in heaps so that diffusion is not rapid. This idea was borne out by the results of an experiment showing the deleterious effect on the seed of contact with the dry, powdered, paraformaldehyde. Dry, untreated seeds were put in Syracuse watch crystals and covered with powdered paraformaldehyde which was packed closely around them. The watch crystals were left uncovered and placed in a dry place. At

intervals 25 seeds were removed and germinated, with the results shown in Table VI.

TABLE VI.—*Percentage of germination of wheat kept in contact with powdered paraformaldehyde* [a]

Length of contact.	Experiment 1, Little Club, harvester-thrashed.	Experiment 2, Little Club.		Experiment 3, Early Baart, hand-thrashed.	Experiment 4, Early Baart, hand-thrashed.	
		Harvester-thrashed.	Hand-thrashed.		Uninjured.	Seed coats broken over embryo.
1½ hours	80					
24 hours	50	50	90	100	90	10
2 days		30	90	80	80	0
3 days	20					
5 days					80	0
6 days		0	70	30		
8 days					70	0
14 days	10	0	0	0		
20 days					30	0
42 days					20	0
Control	100	100	100	100		

[a] No germination tests were made at the intervals represented by blank spaces.

The data in Table VI show that dry paraformaldehyde powder kills seed in contact with it, even those with unbroken seed coats. Those with the testa injured, either by the thrashing machine or by breaking in the laboratory with a needle, were injured and killed most quickly, as was to be expected. It is noteworthy that the appearance and progression of the seed injury was similar to that previously noted as occurring in the successive germinations of treated seed being dried. The first sign of injury was the retardation of the development of the plumule, which became gradually more extreme. Finally, it was so injured that it did not elongate at all after emerging from the seed, the sheath breaking prematurely and showing the same curved, sickle-shaped deformity previously found so characteristic of dried formaldehyde-treated seeds. As it would be difficult to conceive of any absorption of solid paraformaldehyde, the only plausible explanation of such "paraformaldehyde injury" is the penetration of formaldehyde gas through the seed coat, the gas being concentrated in the interstices of the powder as a result of the evaporation of the latter. Later experiments in which it was found that absolutely dry seeds were uninjured by formaldehyde fumes make it appear probable that the gas is dissolved in the cells of the seeds and really diffuses into them as a solution.

HUMIDITY AS THE DETERMINING FACTOR IN SEED INJURY

The first hint that the humidity of the atmosphere surrounding the seeds at the time of drying determined the amount of seed injury from treatment with formaldehyde—by controlling the evaporation of the

solution on the seed and the formation of paraformaldehyde—came from the difference in the degrees of injury sustained by the original samples of treated wheat dried in the greenhouse, laboratory, and refrigerator (see Tables I and II and Pl. 37). The dried seed from the greenhouse, where the atmosphere was warmest and most humid, was the least injured. From our knowledge of the unstable constitution of paraformaldehyde it seemed probable that it would form but slowly if at all in the presence of moisture. Work, therefore, was undertaken to determine whether the degree of this seed injury resulting from drying after treatment depended on the humidity of the atmosphere at the time of drying.

The moisture content of the three dried samples of treated seed from the greenhouse, laboratory, and refrigeratorw as determined after six weeks of storage. By drying the seed to constant weight in an electric oven at a temperature of 95° C. it was found that the seed dried in the laboratory contained 13.28 per cent moisture, that from the refrigerator 15.35 per cent, and that from the greenhouse 16.63 per cent. Samples of each lot were then tested qualitatively by means of Tollen's silver-mirror aldehyde test for the presence of formaldehyde. A distinct difference was obtained. The precipitate appeared most rapidly and was most dense in the laboratory-stored seed which had the small moisture content, while it was decidedly least in the greenhouse-stored sample with highest moisture percentage. These facts then suggested that the formation of paraformaldehyde is dependent on the dryness of the atmosphere. Since all evidence points to the fact that seed injury upon drying after treatment is dependent on the formation of paraformaldehyde on the seeds as the solution evaporates, it follows that seed injury may vary inversely with the moisture content of the surrounding atmosphere. So far as the three seed lots of this original experiment were concerned, this was true, for the greenhouse where least injury occurred was most humid and the laboratory where injury was most extreme was driest. However, more evidence was necessary, and this could be obtained only by storing treated seed under controlled and definitely known moisture conditions.

Atmospheric humidities varying by 10 per cent intervals from saturation over water to dryness over concentrated acid were produced in desiccators by the use of sulphuric acid dilutions.[1] Given the specific gravity of the solutions necessary to produce the desired atmospheres (Pl. 38, B), they are easily made up in quantity by means of specific gravity spindles and kept in stock bottles (*17*, *p. 114*).

[1] Since these experiments were completed, a paper written by Neil E. Stevens (*15*) has come to the writer's attention in which a table is given showing the approximate humidities obtained in desiccators containing aqueous solutions of sulphuric acid of various specific gravities. These differ somewhat from those given by Woodworth (*17*, *p. 114*), and the method is described more fully and the data given are more complete.

Some of the same machine-thrashed Little Club seed used in all these experiments was treated with a 0.1 per cent solution, and, after the surplus liquid was removed by spreading on towels for a half hour, the seed was divided into 11 lots, each lot nearly filling a rectangular glass dish 6 by 8 cm. and 3 cm. deep. One of these dishes of wheat was then placed in each of the 11 desiccators containing 100 cc. of their respective sulphuric acid and water mixtures. These solutions were changed at the end of the first, second, third, fifth, and tenth days, so that they were kept at the proper strength. Samples of wheat were removed after various intervals, and the injury was determined by germinating on blotters at room temperatures.

TABLE VII.—*Relation between seed injury from drying after treatment with a 0.1 per cent formaldehyde solution and the humidity of the atmosphere*

Specific gravity of sulphuric acid and water mixtures.	Approximate percentage of humidity produced in desiccators (20° C.).	Percentage of germination after storage in desiccators for—									
		1 day.	2 days.	5 days.	7 days.	10 days.	16 days.	22 days.	26 days.	28 days.[a]	42 days.
1.000	100	96	98	94	98	(b)	(b)	(b)	(b)	(b)	(b)
1.070	90	98	98	96	96	(b)	(b)	(b)	(b)	(b)	(b)
1.130	80	96	94	92	94	94	96	(b)	(b)	(b)	(b)
1.206	70	94	98	90	90	96	90	98	90	88	100
1.273	60	96	96	.....	90	84	96	90	96	86	92
1.334	50	96	96	.....	82	84	88	86	92	94	88
1.400	40	98	98	.....	70	82	74	78	84	74	82
1.470	30	98	96	.....	74	76	78	70	84	72	76
1.530	20	96	94	74	84	76	80	84	82	70	72
1.604	10	94	98	84	80	80	84	72	88	72	76
1.840	0	96	92	84	88	88	88	82	84	64	86
Control	.....	96	100	98	98	100	98	96	98	100	96

[a] Germinated in soil.
[b] Attacked by molds.

A study of these germination percentages reveals several most interesting facts. It is at once obvious that they show the existence of a close relationship between the seed-treatment injury caused by drying and the humidity of the atmosphere. They show that there is no injury in the damper atmosphere of 70 per cent humidity and above, so long as the seed is not attacked by molds. They show also that there is less injury in the dryest desiccators, those containing from 20 per cent moisture to none at all, than in those of intermediate humidities. These comparative injuries are made clearer by a graph (fig. 2) the points on which represent the averages of all the percentages obtained for each sample, beginning with those obtained after five days' storage. The data for the

samples in 80, 90, and 100 per cent humidities were not included because of the small number of germinations obtained before the seed was partially destroyed by molds. The curve shows graphically that there was a decrease in germination from the uninjured samples in the high humidities to those in 30 per cent humidity, after which it increased in the successively drier desiccators but did not reach normal.

It is also noteworthy, in connection with the data given in Table VII, that no injury appeared, as indicated by the germinated samples, until at some time between two and five days after treatment. Thus, a test of all samples after three days, the results of which were not included in the table because of complications from an unusual growth of Rhizopus in the germinators, showed no visible evidence of formaldehyde injury. The harmful effects were first apparent after five days' storage, where, however, molds again interfered with the germination of four of the samples.

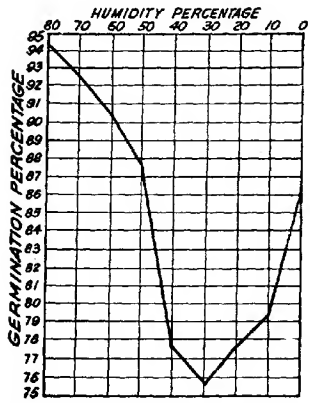

FIG. 2.—Graph showing the relation of humidity of the air to percentage of germination of stored seed in first experiment.

It will also be noticed in Table VII that the successive percentages obtained show no increasing injury between the 5-day and 42-day germinations. They differ in this from those of many other experiments (Tables I to IV).

Some months later this experiment was repeated with some of the same lots of wheat. This second experiment differed from the first, so far as was known, only in the smaller quantities of treated wheat placed in each desiccator and in the amounts of sulphuric acid and water mixtures used. Approximately 20 cc. of wheat were put in each desiccator, which was about one-fourth of the quantity used before. One hundred cc. of the desiccating solutions were left in the desiccators for the first 24 hours, at the end of which period they were changed, and 200 cc. quantities of the fresh solutions were substituted and left unchanged for the rest of the experiment. As will be seen from Table VIII, the resulting seed injury was more extreme than in the first experiment and reached its maximum in a more humid atmosphere (Pl. 39). The explanation for the difference may be the greater or lesser effectiveness of the desiccating solutions, owing to the difference in the quantities used and in the amount of seed dried over each.

The data in Table VIII show, as do those of the preceding experiment, that the highest humidities allow no injury and that in the lowest the germination percentages are normal also, only the retarded growth giving

evidence of some deleterious effect of the treatment. There is a very definite point of maximum injury—the 70 per cent humidity. This is somewhat different from the situation in the preceding experiment, where the maximum injury was at approximately 30 per cent humidity, with none at all occurring at 70 per cent.

TABLE VIII.—*Data from the second experiment on the relation between humidity and seed injury after formaldehyde treatment*

Specific gravity of sulphuric acid and water mixtures.	Approximate percentage of humidity produced in desiccators (20° C.).	Stored 10 days.		Stored 21 days.		Stored 35 days.		Stored 42 days.	
		Germination.	Height of plants.[a]	Germination.	Height of plants.[a]	Germination.	Height of plants.[a]	Germination.	Height of plants.[a]
		Per ct.	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*	*Per ct.*	*Cm.*
1.000	100	96	5	98	8.0	.....	.....	.....	.....
1.130	80	94	5	100	7.0	.....	.....	.....	.....
1.206	70	6	1—	4	1.0—	0	.....	12	1.0—
1.273	60	18	1—	38	1.0—	20	.....	12	1.0—
1.334	50	96	2	70	1.0	45	.....	88	1.5
1.400	40	90	4	84	1.5	80	.....	92	1.5
1.530	20	90	5	100	5.0	90	.....	96	3.5
1.604	10	98	5	98	4.0	80	.....	88	3.5
1.840	0	96	5	98	6.0	100	.....	88	3.5
Control	.....	98	5	98	7.0	100	.....	88	6.0

[a] The average heights of the plumules after 6 days are given for each germinating sample, because a comparison of these for all the samples of any one test shows any injury indicated by retardation which sometimes would not be shown by the germination percentage alone. A height of less than one centimeter (1—) indicates extreme injury, with usually stunted, deformed plumules which could not reach the surface of the soil.

Figure 3 shows more plainly the comparative germinations given in Table VIII. As in figure 2, each point was obtained by averaging all the germination percentages given by the sample stored at each indicated humidity.

Since all germinations were made in blotters without temperature or humidity control, the rate of growth of seedlings of successive 6-day germinations of the same sample varied in a meaningless way and so were valueless except for comparisons of the injury shown by the different samples in the same germination test. However, as noted in the discussion of the first experiment with the desiccators, the growth measurements follow closely the germination percentages and are more delicate indicators of harmful effects of treatment than the latter.

If the averages of the heights of the seedlings from each desiccator for all the germination tests of both experiments be plotted with the humidities in which the respective seed samples were stored, a graph such as figure 4 is obtained. These heights were measured after six and seven days' growth, but the conditions of germination in successive

tests were so variable that accurate comparisons of growth can not be made. However, the graph, in its similarity to the germination graphs, illustrates the close correlation between viability of the sample and the retardation of seedling growth. Being an average of the two experiments, it brings the maximum growth retardation to 60 per cent.

Since the relation between degree of seed injury and the moisture content of the atmosphere in which the seed was stored had been shown, it was surmised that a similar correlation could be shown to exist between humidity and the formation of paraformaldehyde. After the seed samples of the first experiment were removed from the desiccators, a Syracuse watch glass containing 10 cc. of commercial formaldehyde solution was placed in each. The solid polymer first appeared after three days as a white suspension in the dishes in humidities of 20 and 10 per cent, and in the very dry atmosphere over concentrated acid. Two days later only the dry, white solid was left in these dishes, and a white precipitate made the solutions opaque in the 30, 40, and 50 per cent atmospheric humidities. The density of the suspensions was in inverse proportion to the humidity in these desiccators. Not until 10 days had passed did any paraformaldehyde appear in the 60 per cent humidity, at which time all that formed earlier in the dishes in the dryer atmospheres was dry. No sign of the white solid ever appeared in the more humid desiccators, although the solution in 70 per cent eventually evaporated to dryness (Pl. 38, B). It was very interesting thus to find that the highest humidity permitting the formation of paraformaldehyde was also the highest in which seed injury occurred after treatment with the 0.1 per cent solution of formaldehyde—that is, the germination of wheat was lowered in the same desiccators in which paraformaldehyde formed upon the evaporation of formaldehyde solutions in them.

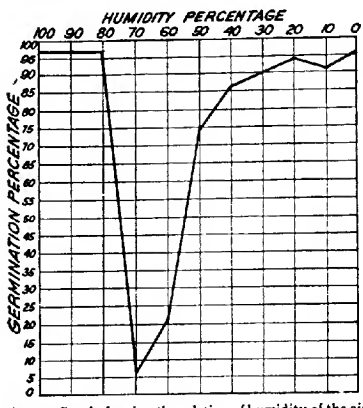

FIG. 3.—Graph showing the relation of humidity of the air to percentage of germination of stored seed in second experiment.

Again, at the end of the second experiment, after the wheat was removed from the desiccators, dishes containing equal quantities of undiluted formaldehyde solutions were placed over the sulphuric acid dilutions, and the appearance and rapidity of formation of paraformaldehyde were noted. In this case 5-cc. instead of 10-cc. quantities were used. After two days, the first white suspension appeared in the desic-

cators having humidities varying from 40 per cent to dryness, being very faint in the former and increasing to a considerable quantity in the latter. The next day a faint opaqueness showed in the dishes of solution in the 50 per cent, and on the day following in those in the 60 per cent humidity, at which time all those in the drier chambers were entirely dry. It is indeed interesting that although no solid ever formed in the 70 per cent humidity, this dish, as in the preceding experiment, evaporated to dryness but left no residue. The volume of liquid left unevaporated in the dishes in the damper atmospheres was greater the higher the humidity (Table IX).

When the residue of paraformaldehyde left after the evaporation of the solutions in the drier desiccators was weighed, it was found in both experiments that, in general, the quantity formed varied inversely with the humidity of the atmosphere (Table IX). Since the degree of injury to the stored treated wheat was in the opposite order, it was at once evident that the factor causing the progressive variation in seed injury in the desiccators was not the quantity of paraformaldehyde formed on the seeds. Before this point is considered further, however, the results of a contemporaneous experiment should be presented. When the dishes of formaldehyde solution were placed in the desiccators to be evaporated, small quantities of untreated seed were inserted at the same time to determine if formaldehyde gas would evaporate in each humidity to produce sufficient concentrations in the different atmospheres to kill the wheat exposed to them. When samples of this wheat were germinated at the end of the experiment, surprising results were obtained. It was found after both experiments that there was no germination of this seed from desiccators of 70 per cent humidity and above and that the germination percentages of seed from the drier atmospheres varied inversely with the moisture percentage, the seed being least injured by the formaldehyde fumes from the solution over concentrated acid. All these secondary experiments on the dependence of the behavior of formaldehyde and its solutions on atmospheric humidity are summarized in Table IX.

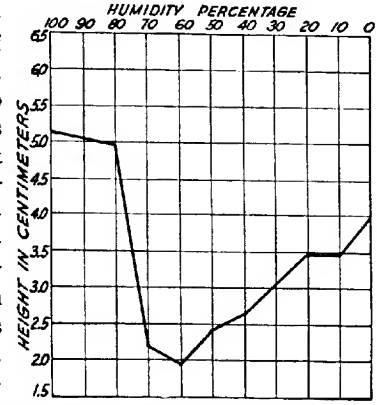

FIG. 4.—Graph showing the relation between humidity of the air and seed injury as indicated by rate of growth of germinated seedlings.

In brief, then, the facts are these: The seed injury resulting after treatment with a 0.1 per cent solution, which occurs as the result of drying

in atmospheres of such moisture content as permit the formation of paraformaldehyde in evaporating solutions, is greatest in intermediate humidities, becoming less as the moisture percentage decreases. This is in spite of the fact that there is an increase in the quantity of paraformaldehyde formed in these successively lower humidities. Secondly, the degree of injury to untreated seed placed in desiccators alongside of evaporating formaldehyde solutions in closed chambers is least in the driest atmosphere and increases with increased humidity. It therefore seems probable that the seeds in the lower humidities were so dry that penetration of the seed coat by formaldehyde was difficult because of the lack of sufficient moisture to permit solution of the gas on or in the testa and its subsequent diffusion to the embryo.

TABLE IX.—*Relation of the humidity of the atmosphere to the evaporation of formaldehyde solutions, the formation of paraformaldehyde, and the effects of the fumes on untreated wheat*

Humidity.	Length of time before appearance of paraformaldehyde in the solutions.		Weight of paraformaldehyde formed.		Volume of solution left unevaporated.		Germination of untreated wheat left in desiccators during evaporation of formaldehyde.	
					After 40 days.	After 24 days.		
	Exp. 1 (10-cc. quantity).	Exp. 2 (5-cc. quantity).	Exp. 1 (10-cc. quantity).	Exp. 2 5-cc. quantity).	Exp. 1 (original volume 10-cc.).	Exp. 2 (original volume 5-cc.).	Exp. 1 (with 10-cc. quantity).	Exp. 2 (with 5-cc. quantity).
Per cent.	*Days.*	*Days.*	*Gm.*	*Gm.*	*Cc.*	*Cc.*	*Per cent.*	*Per cent.*
100	.....	.....	.....	.....	9.6	5.0	0	0
90	.....	.....	.....	.....	7.2	.....	0	0
80	.....	.....	.....	.....	5.4	1.8	0	0
70	.....	.....	.....	.....	.0	.0	0	0
60	10	4	0.07	0.07	.....	.....	0	0
50	5	3	.40	.96	.....	.....	0	0
40	5	2	1.33	1.14	.....	.....	0	0
20	3	2	2.25	1.36	.....	.....	4	10
10	3	2	1.12	1.42	.....	.....	36	16
0	3	2	1.12	1.36	.....	.....	54	30

In presenting this explanation, we are assuming that formaldehyde does not penetrate seed coverings easily, if at all, as a gas but must be dissolved. A small quantity of moisture in the cells of the seed covering therefore would perhaps be necessary to permit injury from formaldehyde fumes. This is consistent with the statement of Humphrey and Potter (7) that—

> disinfection with formaldehyde gas seems to require some moisture.

This supposition would explain the relation found between the degree of injury resulting from drying treated seed and the humidity of the atmosphere in which the seed is dried. With the atmosphere sufficiently dry to allow the formation of the "formaldehyde reservoir"—the coating of paraformaldehyde on the seed—the ease of penetration of the formalde-

hyde gas constantly formed next to the seed by its decomposition would be determined by the moisture in the seed coat. It would follow, as was actually found, that there would be a point where maximum seed injury would occur—at a humidity low enough to permit the solid polymer to form on the seed as the solution evaporates, yet high enough to permit diffusion in solution of the gas formed from it through the cells of the seed coat to the embryo. Thus may be explained the gradual lessening of the degree of injury from the point of maximum injury to practically normal germination in dry atmospheres.

The work of Arcichovskij (1) on the effect of graded concentrations of formaldehyde solutions ranging from 0.125 to 40 per cent supports the assumption that the ease of penetration of formaldehyde is dependent on the dilution of the solution as it passes through the cells into the seed. He found that, for any given duration of exposure, seed injury did not increase directly with the concentration of the solution. After a definite point of maximum injury, the harmful action of the solution decreased with increased concentration, until in all exposures over four hours the undiluted 40 per cent formaldehyde solution caused less injury than the 0.125 per cent dilution. For instance, after 256 hours 37.5 per cent of the seeds from the 40 per cent solution germinated, while those in the 0.125 per cent solution were entirely killed after 32 hours' exposure. The curve he has drawn showing the relation between concentration of the solution and the percentage of germination is similar to the curves in this report which show the relation between humidity and formaldehyde injury to seeds upon drying after treatment.

The preceding paragraphs merely offer a suggestion of an explanation of the observed facts. This interpretation of these facts is based on several assumptions which have not been proved by direct evidence. One is that paraformaldehyde, as a solid, does not injure seeds but only upon its breaking down into formaldehyde gas and forming a toxic vapor about the seed. Another is the assumption that this formaldehyde does not penetrate seed coats as a gas but that it must enter in solution.

It should be pointed out here that in experiment 2 the maximum injury occurred in the atmosphere of 70 per cent humidity (Table VIII) in the desiccator in which it was found that the formaldehyde solution evaporated to dryness without the formation of paraformaldehyde (Table IX). This indicates that seeds may be injured by the concentration of a 0.1 per cent solution on the surface as evaporation proceeds, without the formation of the solid polymer.

RELATION OF DEGREE OF INJURY TO MANNER OF DRYING

In the course of the experiments it was noted that the drying injury was not always of the same severity, and it was finally found that it depended on the aeration of the drying sample, thinly spread seed escaping the injury suffered by that dried in heaps. This observation was

decided to be consistent with our previous conclusions as to the manner in which formaldehyde solutions injure the treated seeds upon which they dry. If injury occurred as the result of the close adherence to the seed of concentrated formaldehyde gas formed by the decomposition of paraformaldehyde deposited on the surface as the seed dried, then it would follow that well-aerated seeds might very probably escape injury by virtue of the rapid breaking down of the polymer and its escape by diffusion into the air. Formaldehyde gas is heavier than air, so that if seeds were dried in large quantities in sacks or in boxes, diffusion would be slow and the air around the seeds would become saturated with gas, which would be held around them long enough to cause seed injury.

The evaporation of but a relatively small quantity of paraformaldehyde in a closed space saturates the atmosphere so that further breaking down

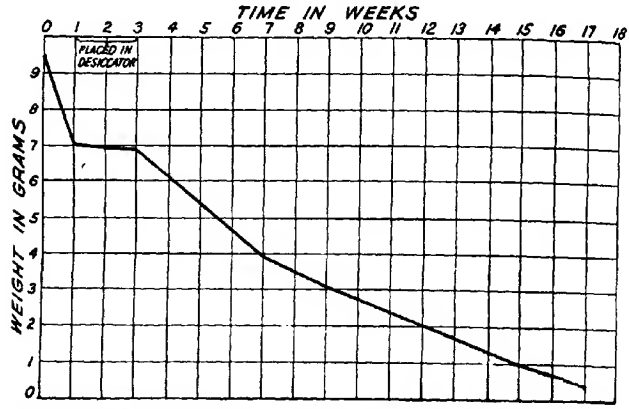

FIG. 5.—Graph showing the diminution in the rate of evaporation of paraformaldehyde inclosed in a desiccator of 2,400-cc. volume.

of the solid is inhibited by the partial pressure of the formaldehyde gas. This was shown experimentally by placing some paraformaldehyde in desiccators at the same time that dishes containing approximately the same quantities were evaporating in the open air of the room. The rate of evaporation of each sample was measured by the loss in weight after successive weekly intervals. Figure 5 illustrates the initial rapid rate of evaporation of a sample in the open air and the slowing up of that rate when it was placed in a 2,400-cc. desiccator containing calcium chlorid as a drying agent. When the sample was removed from the desiccator the rate increased again, and the curve representing this period shows a steady, even fall, until after 18 weeks the solid had practically disappeared. If we compare the curve with figure 1, we note that whereas when the sample is exposed to the open air it disappears entirely, when it is inclosed and hence unaerated its evaporation practically stops. The exact weight

of the solid which when evaporated in a space of 2,400 cc. checked by its partial pressure further decomposition of the sample is not shown. It would appear to be approximately 0.1 gm., the average decrease in weight found upon successive weekly weighings of the inclosed sample. The slight fall of the curve for this period in the desiccator is explained by the fact that when the dish was removed each time for weighing the concentration of gas within would be diluted and so the sample would continue to lose weight. A parallel control experiment gave the same curve and the same total loss in weight, 0.21 gm., during the two weeks in the desiccator.

The significance of this curve for the problem of post-treatment injury of dried seeds is that when there is no aeration the formaldehyde gas from the evaporating paraformaldehyde on the seeds easily saturates the atmosphere in the interstices of the sample and inhibits the evaporation of more of the solid. The slower the outward diffusion of the gas the longer will the paraformaldehyde remain on the seed surfaces and the longer will a toxic atmosphere exist about them. As the penetration of the seed coat and subsequent injury by formaldehyde is comparatively slow, usually occurring in from 3 to 5 days with a 0.1 per cent solution (Table VII), it is entirely conceivable that with rapid drying and thinly spread seed any paraformaldehyde formed can be completely evaporated and its dissipation effected so rapidly that it can not enter and injure the embryo.

Seeds treated with a 0.2 per cent solution, twice as strong as the usual treatment, were dried without injury when spread in a single layer on towels, while such seeds dried in quantity in an open box were practically all killed. That it was the time required for the formaldehyde to penetrate the testas which saved the former lot of seed was shown by the fact that some of the same sample which had the seed coats broken over the embryos were dried beside the others and were severely injured after 24 hours. In the former case the paraformaldehyde evaporated and diffused before it could penetrate the sound seed coat. But when a 4.5 per cent solution was used, even the seeds with unbroken coats were found to be injured after 24 hours' drying under these conditions. The quantity of paraformaldehyde formed presumably was too great to escape before seed injury occurred. The broken seeds dried at the same time showed proportionately greater and more rapid injury than the broken seeds treated with the weaker solutions. It will be noted in Table X that embryos exposed by broken testas are not injured by a 10-minute dip into formaldehyde as strong as 0.2 per cent but that a 4.5 per cent solution is injurious. It is significant that with rapid drying and aeration even the seeds with broken seed coats were not injured by a 0.1 per cent solution. Yet it has been found repeatedly that when perfect seeds thus treated are dried without aerating they are injured or killed.

TABLE X.—*Relation between strength of formaldehyde solution, condition of seed coat, and the cumulative injury to Early Baart wheat well spread during the drying period*[a]

Length of drying period.	4.5 per cent formaldehyde solution.				0.2 per cent formaldehyde solution.				0.1 per cent formaldehyde solution.			
	Seed coats unbroken.		Seed coats broken over embryo.		Seed coats unbroken.		Seed coats broken over embryo.		Seed coats unbroken.		Seed coats broken over embryo.	
	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.
	Per cent.	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*
0	100	5.0	60	2.5	95	5.0	95	5.0	95	5.0	90	4.5
1½ hours	95	2.5	30	1.0—	95	3.5	95	2.5	100	4.0	100	4.0
4 hours	95	2.5	40	1.0—	95	3.5	90	2.5	100	5.0	100	5.0
24 hours	95	2.5	25	1.0—	100	4.0	80	3.0	100	5.0	90	5.0
3 days	75	2.0	20	1.0—	95	2.0	45	1.0	100	5.0	95	5.0
6 days	80	1.0	15	1.0—	95	2.5	45	1.5	100	5.0	95	3.0
14 days	65	1.0—	0	1.0—	95	2.0	10	1.0	90	3.5	100	3.5

[a] The average heights of the plumules after 6 days are given for each germinating sample, because a comparison of these for all the samples of any one test shows any injury indicated by retardation which sometimes would not be shown by the germination percentage alone. A heighth of less than one centimeter (1-) indicates extreme injury, with usually stunted, deformed plumules which could not reach the surface of the soil.

In brief, Table X shows that when treated seed is dried rapidly by being thinly spread in the laboratory, it is uninjured by a 0.1 per cent solution even if the embryos are exposed by broken seed coats; that seed treated with a 0.2 per cent solution is uninjured if the seed coat is perfect, but severely injured after 24 hours if it is broken; and that, with a 4.5 per cent solution, perfect seeds are slowly injured and that seeds with broken testas are injured by the dip into the treating solution, which injury rapidly increases upon drying. The cumulative nature of this seed injury is well shown by the germination data for all these injured samples.

Lest there be any misunderstanding, it may be well to consider again the case of treated seed which is sealed damp. It may be asked at this point that if aeration is necessary to prevent injury from formaldehyde fumes, how can seed stored damp in sealed jars remain uninjured? The answer is probably to be found in the fact that paraformaldehyde does not form on damp seeds; hence the damp seeds are not surrounded by concentrated formaldehyde vapor. The moisture in the jar is a weak dilution, and neither it nor the amount of formaldehyde in the air in the presence of so much water is strong enough to injure the seed. Moreover, the formaldehyde does not remain on damp seeds indefinitely, owing to the activity of microorganisms which decompose it. The case is different with solutions stronger than 0.1 per cent, however. Damp seed is slightly injured by a 0.2 per cent solution after 24 hours' storage, and a 4.5 per cent solution is fatal in a sealed jar. Whether in these instances it is the solution on the seed which injures or the resulting

formaldehyde fumes was not determined; but according to Auerbach and Barschall (*2*), the partial pressure of formaldehyde gas above solutions in a closed space increases with the concentration of the formaldehyde solution; hence the fumes may be the cause of injury.

Several experiments showed clearly the varying degrees of injury resulting from drying the seed at different rates. The usual procedure was to treat some wheat with a 0.2 per cent solution and some barley with a 4.5 per cent solution, the latter being more resistant to drying injury and therefore requiring the use of a strong solution to produce it. Some of each lot was then spread thinly over towels on the laboratory table, while the rest was put in an open tumbler or a slender, uncovered bottle. For comparison, a third lot usually was placed in a similar bottle and sealed while damp. Samples were removed after various intervals and were germinated in the usual way to determine the degree of injury. The data on the germination of wheat are shown in Table XI and those on the germination of barley in Table XII.

TABLE XI.—*Percentage of germination of Little Club wheat treated with 0.2 per cent solution of formaldehyde and dried under different conditions and during periods of varying lengths*

Length of drying period	Experiment 1.			Experiment 2.		Control, untreated.
	Spread on towels.	In open bottle.	In sealed bottle.	Spread on towels.	In open bottle.	
Days.						
0	96	96	96	94	94	96
1	84	74	82	94	76	98
2	.....	.....	.....	98	88	96
3	70	52	84	.....	.....	100
6	64	40	80	76	64	98
18	40	16	82	88	52	98
28	.....	.....	.....	74	52	94
60	50	8	80	56	50	92

Wheat treated with a 0.1 per cent solution was dried overnight in a sealed jar, in an open jar, and in a thin layer on towels. After drying 24 hours, equal samples were washed in equal volumes of water, and the washings were subjected to Tollen's aldehyde test for the presence of formaldehyde. Comparison of the density and rapidity of formation of the silver precipitate showed that there was least formaldehyde on the thinly spread seed and greater amounts on the other two samples. At the end of the second 24-hour period the experiments were repeated. It was found that the amount of formaldehyde on the sealed seed had diminished until it gave a much less dense precipitate than either of the dried samples. Of the latter, the extract from the seed dried in the bottle showed the presence of more formaldehyde than that from the

well-spread seed. Throughout subsequent tests, continued almost daily for two weeks, the dried samples gave stronger reactions than the damp ones, which, after about six days, showed no more than the extract from the untreated control. The dried samples soon gave about equal reactions. The results of the first two tests, which showed that there was more formaldehyde on the seed dried in the open bottle than on that spread on towels, confirm the conclusion already drawn from the germination data—that is, that more paraformaldehyde remained on the seed dried without aeration because the formaldehyde gas could not escape readily from around the seed. Gradually, however, this gas escaped and the quantity present, as shown by the reaction, decreased to that of the aerated sample.

TABLE XII.—*Percentage of germination of Coast barley treated with 4.5 per cent formaldehyde solution and dried under different conditions and for varying periods of time*

Length of drying period.	Experiment 1.				Experiment 2.		
	Spread on towels.	In open bottle.	In sealed bottle.	Control, untreated.	Spread on towels.	In open bottle.	Control, untreated.
Days.							
0	98	98	98	98	80	80	88
1	88	66	64	94	86	74	90
2	.....	.....	.....	.....	82	44	92
6	80	20	4	94	50	0	92
17	68	0	0	90	82	10	96
28	36	0	0	84	52	2	92
42	[a] 80	8	4	96	.....	.....	.....
60	.....	.....	.....	.....	[a] 70	10	90

[a] These increased germinations after 42 days, though they apparently indicate recovery, are probably due to more favorable germination conditions.

From these data it appears that any prediction or explanation of post-treatment injury must be based on the humidity of the atmosphere immediately surrounding the seed and on the manner of drying the seed as affected by its aeration. Temperature may also be an important factor, but its relation to the problem has not yet been determined. Temperature or some other variable must account for the fact that, with all the foregoing conditions controlled, repetitions of experiments do not always give the same results. For instance, in Table XI injury is shown to thinly spread wheat after a subjection to a 0.2 per cent solution of formaldehyde, while less injury is shown in the data in Table X. In these cases the differences may possibly be chargeable to the fact that different samples of different varieties of wheat were used. In one experiment no greater injury occurred to seed dried in an open bottle than to that thinly spread on the table beside it. Such exceptions are only occasional, but they indicate that certain apparently minor factors have not yet been ascertained.

TABLE XIII.—*Germination and seedling growth of Little Club wheat treated with 0.1 per cent formaldehyde solution and sealed in bottles after drying during various periods* [a]

Length of storage period after drying.	Sealed at once after treatment, 21.20 per cent moisture.		Dried 1 hour, 20.26 per cent moisture when sealed.		Dried 10 hours, 17.08 per cent moisture when sealed.		Dried 20 hours, 14.50 per cent moisture when sealed.		Dried 30 hours, 13.81 per cent moisture when sealed.		Dried 48 hours, 12.49 per cent moisture when sealed.		Dried 72 hours, 12.59 per cent moisture when sealed.	
	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.	Germination.	Height of plants.
Days.	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*	*Per cent.*	*Cm.*
0 (end of drying period).....	100	4.5	98	4.5	100	4.5	98	4.5	100	4.5	100	4.5	100	4.0
6.....	100	3.5	98	4.0	84	1.0—	92	1.0—	90	1.0—	94	1.0	98	3.0
12.....	100	5.0	100	5.0	76	1.0—	88	1.0—	98	1.0—	100	3.0	98	4.5
21.....	98	4.5	98	4.0	72	1.0—	52	1.0—	68	1.0—	98	1.5	94	1.5

[a] The average heights of the plumules after 6 days are given for each germinating sample, because a comparison of these for all the samples of any one test shows any injury indicated by retardation which sometimes would not be shown by the germination percentage alone. A height of less than one centimeter (1—) indicates extreme injury, with usually stunted, deformed plumules which could not reach the surface of the soil.

Interesting data were obtained as the result of an experiment originally intended to show the relation between the moisture content of the seed and the degree of injury upon drying. Samples of wheat and barley were treated with a 0.1 per cent solution for 10 minutes, drained 10 minutes, and allowed to dry partially by spreading on towels for an hour. At the end of that time about 70 cc. were sealed in a small screw-top bottle, and the rest were allowed to continue drying. Equal quantities were removed from the drying lot daily for four days and sealed, the object being to get samples of different moisture content so stored as to insure constant humidity in the bottles. It was found that constant weight was reached after two or three days' exposure to laboratory air. The moisture percentage of each sealed sample was obtained by drying in an electric oven at 95° C. Samples from each bottle were germinated after various intervals, and the injury shown by each was compared by means of germination percentages and rate-of-growth observations. Tables XIII and XIV summarize the results of several experiments.

The data in Table XIII show that none of the samples were injured by the drying period which preceded their being sealed. This was due, no doubt, as explained earlier in this paper, to the fact that they were spread thinly and, therefore, were well aerated. In the second place, it shows, surprisingly enough, that the subsequent injury from being sealed did not bear a direct relation to the moisture content of the seed, as had been expected. After 21 days' storage samples sealed wet immediately after treatment and those sealed after 1 hour's drying were uninjured. This was to be expected, for they contained too much moisture to permit the formation of paraformaldehyde. But in all germination tests made after 6 or more days' storage those samples dried for 10, 20, and 30 hours before sealing showed extreme injury, while those dried longer were less injured. Seed dried 72 hours before sealing was nearly as free from injury as the uninjured, damp seed. This lesser injury to the samples dried for the longer periods seemed so puzzling that the experiment was repeated, twice with wheat and once with barley, with the same results.

The data in Table XIV again show that, although no injury resulted from these various drying intervals, yet when the seed was sealed there was extreme injury after 5½, 9, and 24 hours' drying, after 48 hours slight injury (retarded plumules), and after 72 hours practically no injury. The maximum injury occurred in seed dried 5½ and 9 hours, respectively, decreasing steadily with the longer drying periods of the other samples. This shows particularly well in the germinations of seed in soil, where the weak and injured seedlings, called "germinated" on the blotters, did not reach the surface of the ground and so particularly emphasized the injury to the 5½- and 9-hour samples. Elsewhere in this paper it has been noted that blotter germinations suffice to show comparative injuries and to indicate the deformity and retardation of the seedlings; but,

except to one trained to distinguish the weakened and injured seedlings, the germination counts will not give an accurate measure of field results. In soil, the percentage of germination of injured samples will be much lower, of course, depending on the nature of the soil and the difficulty encountered by the seedling in emerging from it.

TABLE XIV.—*Percentage of germination of Little Club wheat and Coast barley treated with 0.1 per cent formaldehyde solution and sealed in bottles after drying for various periods*

Length of storage period after drying.	Dried 1 hour, 20.96 per cent moisture when sealed.	Dried 5½ hours, 18.36 per cent moisture when sealed.	Dried 9 hours, 16.34 per cent moisture when sealed.	Dried 24 hours, 14.81 per cent moisture when sealed.	Dried 48 hours, 12.94 per cent moisture when sealed.	Dried 72 hours, 13.37 per cent moisture when sealed.	Dried 96 hours, 12.15 per cent moisture when sealed.	Control, untreated, 12.06 per cent, moisture when sealed.
LITTLE CLUB WHEAT								
Days.								
1	100	96	88	98	98	100	98	96
7	100	58	60	82	94	94	96	96
7[a]	92	14	2	70	88	100	88	96
14	92	48	42	74	92	92	94	100
COAST BARLEY								
1	94	92	88	92	98	100	96	96
7	76	74	68	92	.....	90	78	.....
7[a]	98	44	66	88	96	100	94	96
14	48	26	20	72	42	66	.....	.....

[a] Germinated in soil; all others germinated on blotters.

This second experiment also demonstrated that this phenomenon is shown by barley as well as wheat. For barley, as for wheat, the maximum injury was to those samples dried for 5½ and 9 hours, with decreased injury to the samples dried longer before sealing.

In subsequent experiments on wheat treated with both 0.1 per cent and 0.2 per cent solution, then dried and sealed, there was always this upward gradation in injury from a maximum below 24 hours of drying to almost normal germination in samples dried for several days and then sealed. However, in the experiment illustrated in Plate 40, there was severe, though lessened, injury to the sample dried three days before being sealed in the bottles.

For a long time after the first results of this nature were obtained they seemed inexplicable. After the later studies of the behavior of formaldehyde and the manner in which it injures seeds through the volatilizing of its polymer, paraformaldehyde, an explanation suggested itself. In the first place, it is obvious from what we know of paraformaldehyde that it did not form on the dampest seeds. Hence, those seeds sealed after one hour showed no injury because at the end of that time they were still damp. Paraformaldehyde formed on those dried more thoroughly, and the gas resulting from its evaporation at once began to diffuse away from around the seeds because they were thinly spread.

As a result of this steady evaporation of the paraformaldehyde from these seeds those spread the longest before sealing had the smallest quantity on them when put in the bottles, while those sealed earlier had increasingly greater quantities. Since evaporation of the solid would continue to a certain extent after the seeds were in the bottles, it would seem plausible that the concentration of formaldehyde gas in the atmospheres of the sealed bottles would vary, being greatest where seed had previously dried for but a few hours and least where it had had a longer time to dissipate into the air before sealing. It follows that the seed injury in each bottle is proportionate to the quantity of paraformaldehyde left on the seed at the time of sealing, which, upon evaporation in the bottle, cannot escape and is held around the seed.

SUSCEPTIBILITY OF OTHER GRAINS TO POST-TREATMENT INJURY

In laboratory experiments it was found that barley is much less sensitive than wheat to dry-storage injury after treatment with a 0.1 per cent solution and often escapes injury altogether. Retardation or a slight lowering of the germination percentage usually results, however, from drying the seed in bulk or from sowing it in dry soil. In experiments where the seed was allowed to lie in dry soil for varying intervals one experiment showed rather severe injury, while the two repetitions showed none at all. If a 0.2 per cent solution or a 4.5 per cent solution is used the characteristic cumulative post-treatment injury occurs markedly, just as in wheat. The latter strength is especially destructive when the seed dries (Table XII). The germination percentages shown in Table XV (on blotters, with one exception) are typical of the results obtained in the laboratory when Coast barley was dried in tumblers after treatment.

TABLE XV.—*Percentage of germination shown by Coast barley when dried in the laboratory after formaldehyde treatment*

Length of drying period.	0.1 per cent solution.		0.2 per cent solution.		4.5 per cent solution, Exp. 2.	Control, untreated.	
	Exp. 1.	Exp. 2.	Exp. 1.	Exp. 2.		Exp. 1.	Exp. 2.
½ hour	94	92	92	94	98	96	90
7 days	90	94	74	86	52	90	88
21 days	84	.....	82	.....	.....	90	.....
42 days	84	.....	32	.....	.....	92	.....
56 days	88	80	70	52	6	90	90
70 days [a]	76	88	34	40	2	.....	92

[a] Germinated in soil.

The presence of the glumes on the barley grains probably affords the protection which makes them more resistant than wheat to the harmful effects of treatment and subsequent drying.

Three sorghums, Brown durra, Honey sorgo, and Sudan grass, were found to be uninjured by either a 0.1 per cent or a 0.2 per cent solution of formaldehyde even after weeks of drying. When the seed was stored dry in the same manner as was the severely injured wheat, no effects of the treatment ever appeared. This probably is due to protection afforded by the glumes in some instances, and in others by the thick seed coats.

PREVENTION OF POST-TREATMENT INJURY RESULTING FROM DRYING

McAlpine (*11*) thought that soaking the seed which had been held some time before sowing prevented the appearance of formaldehyde injury, but neither Darnell-Smith and Carne (*5*) nor Kiessling (*9*) was able to confirm this. The writer also has been unable to show that the injury can be avoided in this way. Soaking the seed hastened the germination, as it always does even with untreated wheat. But the characteristic injury to the seedling remained, and the percentage of germination, although occasionally somewhat augmented, was far from normal. It seems probable, therefore, that the hardening of the pericarp is not the primary injury.

It has been shown in this paper that thorough aeration of the treated seed as it dries retards and lessens storage injury but does not always prevent it (Tables X, XI, and XII). Neither is rapid drying possible where large quantities of wheat are handled. However, it was found that dry-storage injury can be entirely avoided by simply washing the seed with water after treatment (Pl. 41). The extent to which this simple procedure would do away with the danger in the use of formaldehyde solutions is shown by the data in Table XVI.

TABLE XVI.—*Percentage of germination of wheat treated with 0.1 and 0.2 per cent formaldehyde solutions and washed with water, compared with percentage of germination of unwashed samples*

Length of drying period.	0.1 per cent solution.		0.2 per cent solution.		Control, untreated.[a]
	Seed not washed in water.	Seed washed in water.	Seed not washed in water.	Seed washed in water.	
Days.					
0	78	78	72	76	70
7	62	74	50	74	72
14	58	74	30	82	76
30	52	.....	32	76	72
60	36	74	8	72	74

[a] This seed had been injured by fumigation with carbon bisulfid, hence the low germination of the untreated control and washed samples.

SUMMARY

(1) No seed injury was produced by treating wheat with either a 0.1 per cent (1 to 40) or a 0.2 per cent (1 to 20) solution of formaldehyde if the seed was germinated immediately after treatment.

(2) If treated seed is held several days or more before sowing, it is severely injured if allowed to dry without thorough aeration during the storage period. If, however, the seed remains damp, it suffers no injury from a 0.1 per cent solution and can be so kept indefinitely or until attacked by molds.

(3) Post-treatment injury is usually cumulative, increasing in degree the longer the seed is stored.

(4) This seed injury upon drying apparently is due to a deposit of paraformaldehyde on the seed, which forms as the formaldehyde solution evaporates. The solid paraformaldehyde, being volatile, is constantly breaking down into formaldehyde gas. This gas, being thus concentrated and held so close to the seed, penetrates it slowly, probably going into solution in the testa.

(5) The degree of post-treatment injury depends primarily on atmospheric humidity during the storage period. In atmospheres damper than 70 per cent humidity the treated seed can be kept indefinitely without ill effects. In those of 70 per cent and less there is decided injury, which is most severe in the intermediate humidities, gradually decreasing in the lower ones until seed stored in an absolutely dry chamber is almost uninjured.

(6) No paraformaldehyde formed upon the evaporation of formaldehyde solutions placed in these damper chambers in which no seed injury occurred, but it did form in all solutions evaporated in desiccators of 60 per cent humidity and less, the quantities by weight increasing as the atmosphere became drier. Therefore, seed injury in the desiccators was not determined by the quantity of paraformaldehyde formed on the seeds in each.

(7) Untreated wheat, when placed in desiccators of varying atmospheric humidities alongside of evaporating, undiluted 36.2 per cent formaldehyde solutions, was least injured in the absolutely dry chamber and was entirely killed by the formaldehyde vapor in all the chambers damper than 30 per cent humidity.

(8) In view of the facts that treated seed is less injured in very dry atmospheres than in intermediate ones and that untreated seed is least injured by formaldehyde fumes in the dry atmosphere of desiccators, it is considered probable that formaldehyde does not enter seeds as a gas or in the solid polymeric form but in solution in the seed coats. For the maximum seed injury to occur as a result of drying after formaldehyde treatment, therefore, there must be an optimum atmospheric humidity

to permit, first, the formation of paraformaldehyde, and second, the solution of formaldehyde gas in the seed.

(9) This post-treatment injury is minimized by spreading the seed as it dries so that maximum aeration occurs, thus hastening the evaporation of paraformaldehyde and the escape of the gas from around the seed.

(10) Barley is less susceptible to post-treatment injury upon drying after soaking in a 0.1 per cent solution, probably because of the protection afforded by the glumes; but when stronger solutions are used the injury is very severe.

(11) Seed dried for an hour by being thinly spread on towels in the laboratory and then sealed in bottles is uninjured after weeks of storage; but seed dried longer, although uninjured by the rapid drying, is injured upon being sealed, presumably because of the concentration of gas in the bottle as a result of decomposition of the paraformaldehyde on the seed. Treated seed dried from 5 to 24 hours was more injured upon being sealed than when dried for a longer time.

(12) The sorghums, Brown durra, Honey sorgo, and Sudan grass, are uninjured upon being stored dry after treatment, even when a 0.2 per cent solution is used.

(13) Post-treatment injury from dry storage is entirely prevented by washing the seed with water immediately after treatment.

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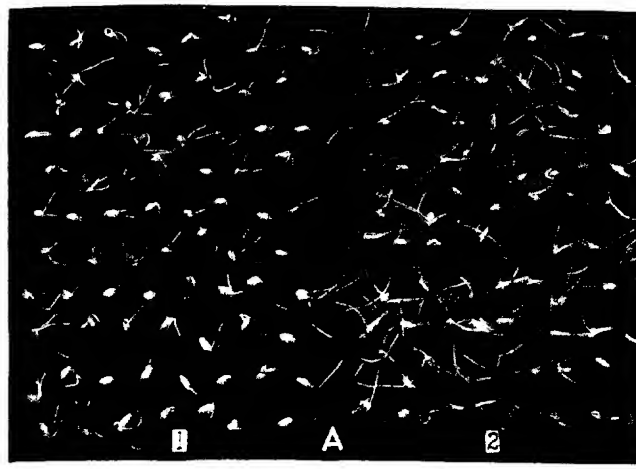
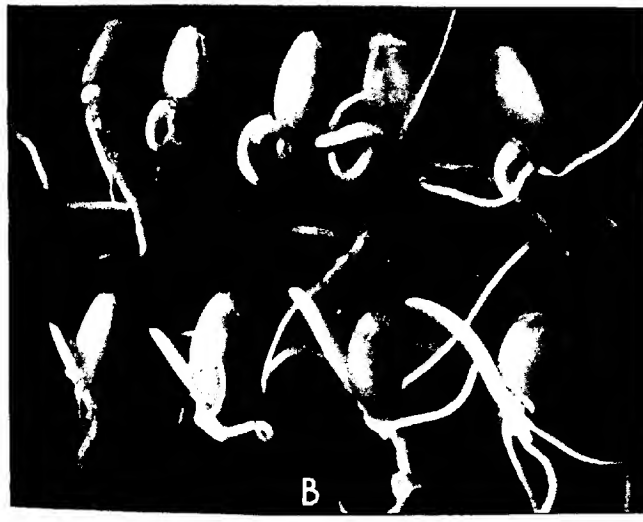
PLATE 36

A.—Post-treatment seed injury occurring when wheat is dried after treatment with a 0.1 per cent solution. Sample No. 1 was stored dry during the 28 days preceding this germination test, and sample No. 2 was stored damp in a sealed jar, the latter germinating at the end of that time as well as the untreated control.

B.—Germinating seedlings of Little Club wheat, showing characteristic post-treatment injury when seed is treated with a 0.1 per cent solution. The upper row shows the usual deformity—curved, sickle-shaped plumule and prematurely broken sheath. Below are seedlings from untreated seed, showing normal germination.


1
A
2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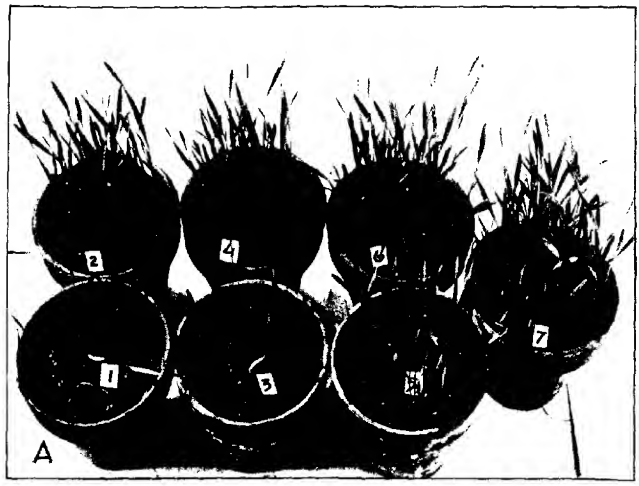
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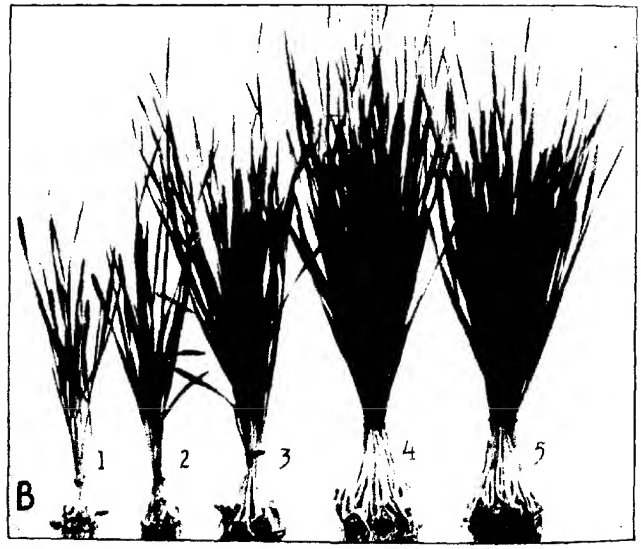
B
1
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3
4
5

PLATE 37

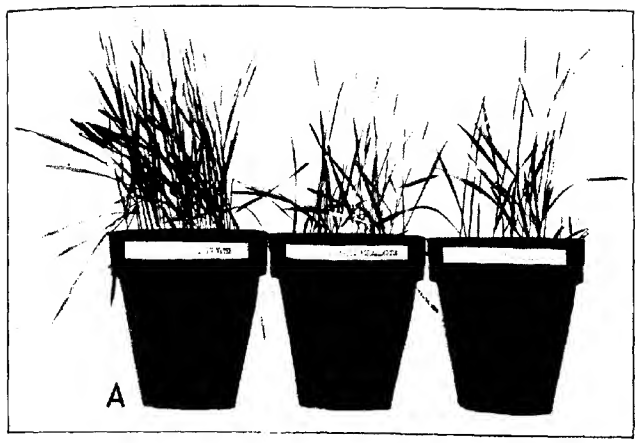
A.—Pots showing germination of treated seed stored for 32 days after disinfection with a 0.1 per cent solution of formaldehyde: No. 1, stored dry in laboratory; No. 2, stored damp in laboratory; No. 3, stored dry in refrigerator; No. 4, stored damp in refrigerator; No. 5, stored dry in greenhouse; No. 6, stored damp in greenhouse; No. 7, control, untreated.

B.—Wheat plants grown in soil from seed stored for 60 days after disinfection with a 0.1 per cent solution of formaldehyde: No. 1, stored dry in refrigerator, germination 18 per cent; No. 2, stored dry in laboratory, germination 34 per cent; No. 3, stored dry in greenhouse, germination 70 per cent; No. 4, stored damp in greenhouse, germination 100 per cent; No. 5, control, untreated, germination 100 per cent.

PLATE 38

A.—Wheat seedlings showing injury produced by allowing the seed to lie in dry soil for 30 days after treatment with a 0.1 per cent solution of formaldehyde: Left, control, dipped in water, 100 per cent germination; center, dipped in 1 to 320 (0.1 per cent) formaldehyde, 62 per cent germination; right, dipped in 1 to 160 (0.2 per cent) formaldehyde, 48 per cent germination.

B.—Desiccators with different degrees of atmospheric humidity obtained by the use of mixtures of sulphuric acid and water in different proportions. The dishes containing formaldehyde were not placed in the desiccators until after the degree of injury to the treated seeds had been determined. The atmospheric humidities were as follows: No. 1, saturated; No. 2, 90 per cent; No. 3, 80 per cent; No. 4, 70 per cent; No. 5, 60 per cent; No. 6, 50 per cent; No. 7, 40 per cent; No. 8, 30 per cent; No. 9, 20 per cent; No. 10, 10 per cent; while No. 11 was absolutely dry, over undiluted acid. Note the white paraformaldehyde formed in these dishes in the drier chambers, beginning with No. 5. (See Table VII for specific gravity readings of sulphuric acid and water mixtures.)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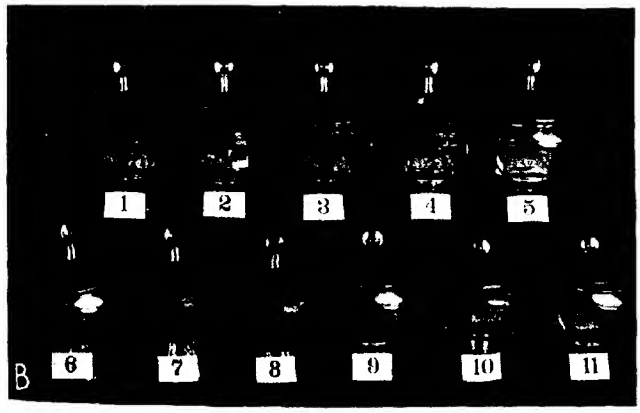
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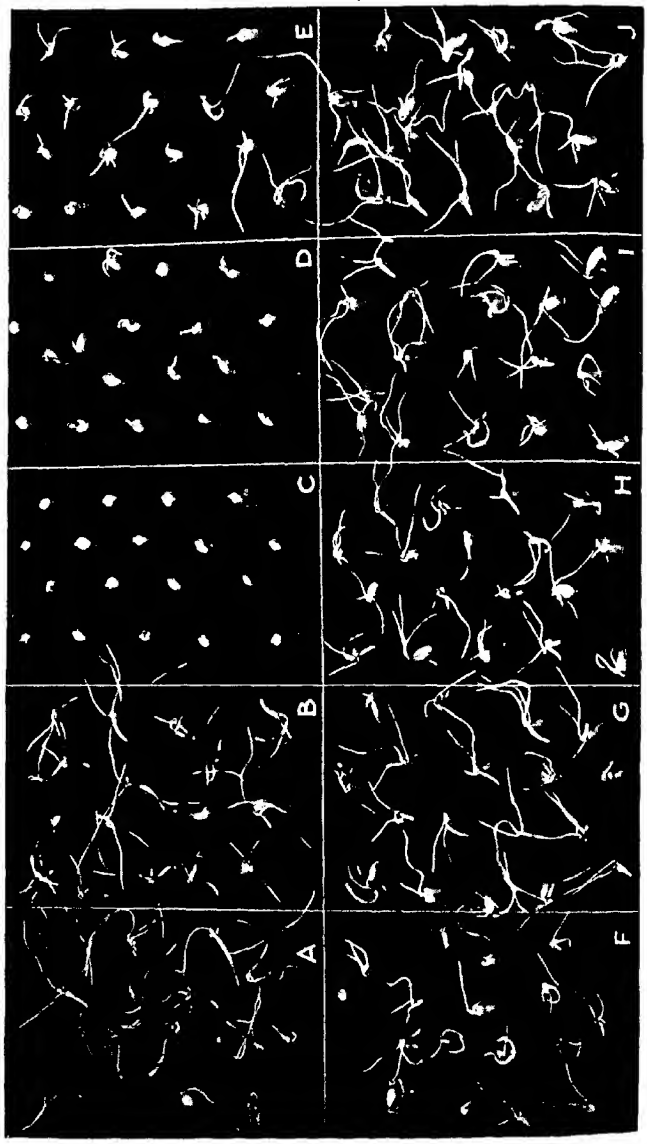

E
J
D
I
C
H
B
G
A
F

PLATE 39

Germinating samples of wheat stored for 35 days after treatment in the desiccators shown in Plate 38 B, illustrating the relation of seed injury to humidity.

Sample A, 100 per cent humidity, 95 per cent germination.
Sample B, 80 per cent humidity, 100 per cent germination.
Sample C, 70 per cent humidity, 0 per cent germination.
Sample D, 60 per cent humidity, 20 per cent germination.
Sample E, 50 per cent humidity, 45 per cent germination.
Sample F, 40 per cent humidity, 80 per cent germination.
Sample G, 20 per cent humidity, 90 per cent germination.
Sample H, 10 per cent humidity, 80 per cent germination.
Sample I, 0 per cent humidity, 100 per cent germination.
Sample J, control, untreated, 100 per cent germination.

PLATE 40

Varying injury to wheat treated with a 0.1 per cent solution of formaldehyde, and stored in sealed bottles:

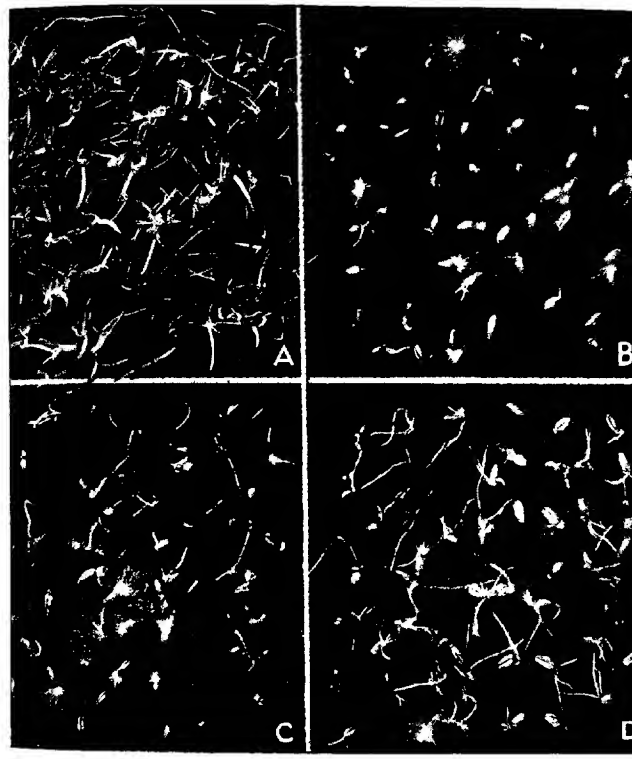
A.—Sealed immediately after treatment, 100 per cent germination.

B.—Sealed after drying 7 hours, spread on towels in laboratory, no germination.

C.—Sealed after drying 24 hours, spread on towels in laboratory, no germination.

D.—Sealed after drying 3 days, spread on towels in laboratory, 14 per cent germination.

The control germinated 96 per cent.


A
B
C
D

Effect of Drying Disinfected Seed Wheat

PLATE 41

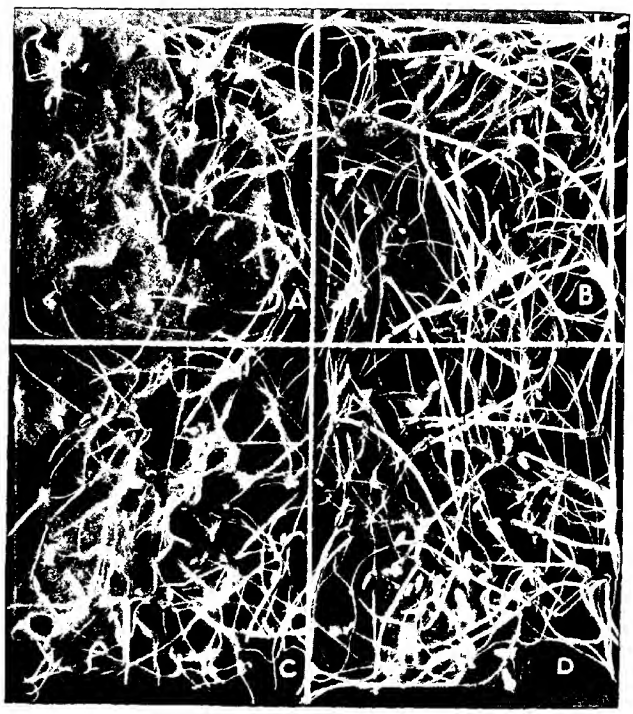


PLATE 41

Germinating wheat kernels, showing the prevention of post-treatment injury by washing the seed with water immediately after treatment. Susceptibility of seeds injured by treatment to Rhizopus and other saprophytes is also shown. This seed had been kept in open tumblers for 30 days after treatment.

A.—Treated with 0.2 per cent solution, which was not washed off before drying, 32 per cent germination.

B.—Treated with 0.2 per cent solution, which was washed off before drying, 76 per cent germination.

C.—Treated with 0.1 per cent solution, which was not washed off before drying, 52 per cent germination.

D.—Treated with 0.1 per cent solution, which was washed off before drying, 74 per cent germination.

Control germinated 74 per cent.

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